A Summary of Current Program, 7/1/64 and Preliminary Report of Progress for 7/1/63 to 6/30/64

WESTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

U.S. AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1963 and June 30, 1964. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California.

UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

Utilization research in agriculture deals with the discovery and development of new and improved products and the invention or perfection of processing technologies. The scientists, engineers, and technologists who carry on this research devote much of their effort to basic studies of physical and chemical properties of agricultural commodities and products derived from them, in order to provide a firm base of exact knowledge for applied developments.

The present report summarizes the current research program of the Western Utilization Research and Development Division (one of four Utilization Divisions in the Agricultural Research Service) and of the State Agricultural Experiment Stations in the areas reported, makes a report of progress toward the objectives of the Federal program during Fiscal Year 1964, and describes a few of the more significant recent accomplishments of this work.

Research Area Covered by this Report

The farm commodities dealt with in this report are the cereal grains, wheat, rice, and barley; alfalfa and other forage crops; wool and mohair; citrus, apples, other fruits, and tree nuts; potatoes and other vegetables and dry beans and peas; castor and safflower; sugar beets; new and replacement crops; and poultry and eggs. Some phases of research on certain of these commodities are pursued in other Utilization Research Divisions than the Western Division: Research on industrial uses of wheat and on milling technology is carried on in the Northern Division; certain areas of research on deciduous fruits and on potatoes and other vegetables are handled by the Eastern Division; particular lines of research on rice, vegetables, and fruits are carried on in the Southern Division. Research on new and replacement crops is carried on in all four Utilization Divisions.

Pharmacological research for all four of the Utilization Research Divisions is conducted at the Albany laboratory of the Western Division, and is described in this report.

Aims of Research on These Commodities

The group of commodities discussed here provides the nation with more than half of its food, either directly (cereal grains, fruits and vegetables, poultry meat and eggs, and beet sugar) or indirectly through feeding of meat animals (forage crops, wheat, barley). The other commodities in the group supply us with our most important animal fibers (wool and mohair) and offer opportunities of development into numberless industrial products (castor, safflower, and other oilseeds).

The general aim of utilization research on both of the two broad categories of farm commodities is essentially the same--to broaden and extend utilization of the commodities and thereby help to stabilize or increase the demand

for them. The scientific procedures of research are broadly the same in both areas, whereas the technologies are in many respects different, especially as between the food materials and all the others. The fundamental justification for carrying on a publicly supported program of utilization research on food products follows a somewhat different line of reasoning than the justification for research to extend the utilization of non-food commodities.

Research toward utilization of non-food products may be based on the avowed public policy of assisting farmers threatened with loss of markets as a result of the swift rise of non-agricultural synthetics, as in the case of wool growers; or on the possibility of developing demand for a presently minor crop to the point where it can be grown profitably on a very large scale in order to remove some of the pressure of surplus from other crops--for example, the development of castor and certain other industrial oilseeds which take land out of cotton and feed grain production.

Research on the processing of farm products for food, on the other hand, is justified primarily by its direct benefit to the entire population through improved nutrition and well-being, reduction of economic losses resulting from spoilage and waste, and increased opportunity to find profitable markets abroad. Indirectly, too, advances in technology through food processing research bring about major and desirable shifts in the commodity supply and demand picture for the country as a whole, as for example in the economical conversion of abundant feed grains into broiler-type chickens, marketable in refrigerated or frozen form throughout the nation and stabilization of dehydrated alfalfa that makes it suitable for export.

Organization of the Division

Research and development along these diverse lines are carried on for the Western Division by a staff headquartered in the Western Regional Research Laboratory, Albany, California. A smaller Department-owned laboratory is operated in Pasadena, California; laboratory space and facilities in Prosser and Puyallup, Washington, are utilized through a cooperative arrangement with Washington State University, Institute of Agricultural Sciences; and laboratory space and facilities in Honolulu, through a cooperative arrangement with the University of Hawaii.

The Albany research staff is organized into six commodity-oriented Laboratories (Cereals, Field Crops, Fruit, Poultry Products, Vegetables, and Wool and Mohair); two functional Laboratories (Pharmacology, and Engineering and Development); and a Pioneering Laboratory concerned with basic studies of plant enzymes. The staff at Pasadena is organized as the Subtropical Fruit Laboratory. The Western Regional Research Laboratory at Albany also houses the Division Director's staff, the staff required for Administrative support of the Division, and that responsible for Plant Management--that is, operation of the buildings, facilities, and grounds.

Division scientists and engineers not only conduct or supervise research in their own experimental facilities, but also greatly extend the scope and influence of their work by planning and supervising developmental activities carried on by cooperating private firms, processor organizations, or industry groups, and by arranging for research by well-qualified scientists elsewhere under research contracts. In addition, certain grants of research funds are placed with investigators in foreign countries; the cost of these foreign research efforts on behalf of American agricultural interests is borne by Public Law 480 funds.

Examples of Recent Accomplishments of the Western Utilization Research and Development Division

New Light-Colored Bulgur. A bland, light-colored bulgur product, low in fiber and in cost, has been developed for use in certain world markets where the darker color of regular bulgur hinders establishment of commercial markets. Complete removal of colored bran layers from cooked hard red winter wheats by an inexpensive lye-peeling technique permits retention of good nutritive properties without staining of inner parts of the kernel by the bran pigments. In either whole kernel or cracked form, the new product is quick cooking and suitable for both the domestic convenience-in-use market and the expanding export markets. Conversion of present bulgur processes to make the new product is neither difficult nor expensive; added costs are estimated at less than one-half cent per pound. Bulgur production costs now run about 5.5 cents per pound. In the past three years export shipments have increased from about 60 million pounds to more than 360 million pounds per year, and this new product provides another entry into large potential new markets for U. S. wheats.

Separation of Dehydrated Forages into High and Low Fiber Fractions. Department scientists carrying out research cooperatively with the Nebraska State Department of Agriculture have developed methods for the dry separation of dehydrated forages into two products. One of these is high in protein, low in fiber, and particularly suited to the dietary requirements of poultry and swine. The other product contains relatively less protein and more fiber, and is better suited for ruminant use. By appropriate adjustments, intermediate products can be obtained to meet any specific market needs. This development opens another avenue to tailor-made feeds to fit specific animal requirements and has excited broad industrial interest. Although evaluation studies are still incomplete, one company has already installed large-scale test equipment to convert part of their 1964 production to dual products. Other dehydrators have expressed enthusiasm for the system, and one concern is reported to be building a new plant which will emphasize dual product production.

WURLAN-Treated Wool Yarn in Commercial Production. Three U. S. companies began this past year producing yarns treated with the ARS process for shrink-proofing wool fabrics. This "WURLANized" yarn is used for knit goods that combine the natural advantages of wool with the easy-care performance of

synthetics, plus other desirable properties important to consumers. Producers claim that these yarns are "dependably washable by any normal method, truly resistant to shrinking and felting, more resistant to abrasion, have improved tensile strength, retain the look and feel of wool, and exhibit no weight loss and no loss in chemical resistance." WURLAN-treated fabrics are being produced at a steadily increasing rate, which is now well over one million yards annually. The extension of the treatment to yarn greatly expands the application of the Department's discovery to a wide variety of knitted structures which otherwise could not be treated. The WURLAN treatment is thus playing an important role in improving the competitive position of wool through providing goods which are machine-washable.

Better Frozen Fruits and Vegetables. While conventional freezing processes usually preserve the flavor, color, and nutritive value of frozen fruits and vegetables, they often have an adverse effect on texture. In common commercial practice freezing requires from 15 minutes to many hours. The scientists found that if freezing is accomplished very rapidly--in a minute or less--such damage does not occur. Green beans frozen by intermittent immersion in liquid nitrogen, for example, have a texture essentially the same as that of fresh beans. Freezing with liquid nitrogen is probably too costly for all but high-valued products. However, other less expensive ways of achieving very rapid freezing are available and they will undoubtedly come into much wider use now that the improvement in product quality has been demonstrated. Retention of fresh-product texture in the processed product will expand the market for frozen fruits and vegetables.

Flavoring Constituent Identified in Grapefruit. Department scientists have discovered the identity of a constituent, nootkatone, in the essential oil of desert grapefruit which appears to be characteristic of the fresh fruit aroma. Nootkatone is present in good quality grapefruit oil to the extent of 0.3%, whereas poorer quality oils contain lesser amounts. It is present in small amounts in certain other citrus oils such as bergamot, lemon, lime, orange and tangerine. There are several potential applications of this discovery: (1) the nootkatone content of grapefruit oil may be used as an objective measure of its flavor quality; (2) addition of nootkatone to grapefruit products should prove to be a simple way to intensify the desired grapefruit aroma. Horticulturists and plant breeders should be able to use the nootkatone content to assess the value of new cultural practices and breeding studies on grapefruit flavor.

New Diffusion Procedure Saves Beet Sugar. A new system for diffusion of beet sugar from cossettes has been developed by Department scientists which promises to increase the efficiency of sugar manufacture. In an effort to minimize stream and ground water pollution, beet sugar factories must return pulp press water to their diffusion batteries. It has been found that by adding the stream of pulp press water toward the head-end of the diffusion battery and the other make-up water at the tail-end, savings of the pulp press water sugar can be made. It has been estimated that 80% of the press water sugar can be saved, resulting in industry-wide savings of sugar worth

$4,800,000. Another application of these principles is in areas where saline well water is used for diffusion of beets. Efficiencies in processing can be enhanced by adding the well water at the tail-end of the diffusion battery and pure water toward the head-end. Savings of sugar, which would otherwise pass into molasses, due to excessive salts, is estimated to be up to $50,000 per year for each of several American beet sugar factories.

Process Developed for Destroying Salmonella in Liquid Egg White. A process has been developed by Department scientists for stabilizing liquid egg white so that it can withstand pasteurization temperatures of 140-143° for 3½ minutes--a condition necessary for destruction of Salmonella. Previously, adequate pasteurization was not possible because the necessary high temperatures cause coagulation of the egg white. The new process entails treatment of egg white with trace amounts of an edible aluminum salt to a concentration of 30 parts per million. Such stabilized pasteurized egg white yields angel cakes of volume and texture equivalent to those prepared with fresh eggs. Successful commercial runs with the new process have been made in two egg processing plants. Nearly 200 million pounds of egg white with a market value of about $25 million are produced annually in the U.S. The development of this pasteurization treatment is an important step toward the elimination of Salmonellosis food poisoning outbreaks that can be attributed to egg products.

Radiation Preservation of Poultry Meat. Department scientists have defined processing factors that minimize deleterious changes in radiation-sterilized poultry meat. The research, supported by funds transferred from the Quartermaster Research and Engineering Command of the Department of Defense, demonstrated the importance of heating poultry to about 180° F. prior to irradiation. In addition to preventing enzymatic reactions resulting in off-flavor, this heat treatment eliminates an unattractive red discoloration which otherwise develops during storage of irradiated poultry. Additional improvement in flavor was accomplished by irradiation at sub-freezing rather than ordinary temperatures. As a result of these and other findings, the QMC has concluded that radiation-sterilized poultry meat is a suitable product for feeding troops in locations where refrigeration is not available or economical.

Discoloration of Potatoes. The tendency for the cut surfaces of potatoes to turn dark when exposed to air often lowers the quality of processed potato products, particularly prepeeled potatoes. Although darkening can be retarded by treating the tissue with sulfites, this may introduce an off-flavor that is objectionable to some people. Public Law 480-supported studies have shed new light on the phenomenon of darkening and suggest a new approach to solution of the problem. It was found that the major factor determining the rate of discoloration is the concentration of the compound, tyrosine, in the tuber and that the tyrosine level is largely influenced by the amount of water available to the growing plant and to a lesser extent by certain other cultural factors. Modification of irrigation schedules and other farming practices may yield potatoes much less susceptible to darkening. This will make possible the development of a consumer retail market for prepeeled potatoes which until now have only been sold to institutional users because of their relatively short storage life.

AREA NO. 1. WHEAT AND BARLEY-- FOOD AND FEED PRODUCTS AND PROCESSING

Problem. Markets for U.S. wheat are not large enough to use our production capacity of about 2.0 billion bushels. Government-regulated curtailment to about 45 of the 70 million acres available allows production of only about 1.2 billion bushels and restricts agricultural strength, which is basic to our economy. Except with the unusual export of wheat in 1963-64, even our curtailed production can be expected to accumulate a costly, government-held surplus at a rate of about 0.1 to 0.2 billion bushels per year. Wheat production can be increased without surplus accumulation only by increasing markets. The domestic food market of 0.5 billion bushels is the most valuable to agriculture and must be protected against changing consumer tastes and new products. Commercial exports of about 0.3 to 0.4 billion bushels (except 1963-64) strengthen agriculture and provide dollar credits against balance-of-payments deficits which have plagued us in recent years. The export donations and concessional sales of 0.3 to 0.5 billion bushels provide food where it is most needed in the world and serve immediate Defense and State Department missions as well as the long-range market development for U.S. agriculture. The efficient conversion of wheat, mill feeds, and barley to meat offers an opportunity to utilize surplus, albeit at lower return to growers than from food products. Processes and products for all market areas must be developed so they will increase and provide an opportunity for the U.S. to expand wheat production. The foundation of scientific knowledge about wheat composition and processing must be expanded by basic research to support product and process developments.

USDA AND COOPERATIVE PROGRAM

Research on utilization of wheat and barley for food and feed seeks to solve the most urgent problems hindering the development of markets for the full productive capacity of U.S. agriculture. The emphasis is on (1) expansion of existing overseas dollar markets; (2) development of wheat food products that will build up long-term markets in developing nations; (3) increasing domestic markets for wheat foods by increased variety, quality, and convenience; and (4) finding means to upgrade mill by-products and barley nutritionally so they will be more valuable as livestock feeds. Basic research on chemical properties of wheat and barley and on their physical properties supports the problem-solving projects of the program.

The baking quality of wheat depends upon the amount and kind of proteins in it, but how the proteins work is still uncertain. Basic chemical studies are conducted on wheat proteins and on the lipids and carbohydrates that interact with proteins to help overcome the uncertainty of predicting baking quality. Chemical changes occur in proteins during the mixing of doughs and we investigate these changes to learn why and how wheat samples differ from one another and how formulas and mixing procedures affect bread quality.

Quality control in continuous mixing equipment, which is now coming into wide commercial use, demands more accurate knowledge of what a given flour will do. The development of overseas dollar markets for wheat are also concerned with bread quality because our most important export markets are in Western Europe and Japan where strong bread wheats are in substantial demand. Investigations on the maturation of hard red winter (HRW) wheat flours are conducted to make them more competitive with hard red spring (HRS) wheats which are less plentiful in the U.S. but abundant in very good quality from Canada, which provides major competition in international trade. Other export markets are expected for new wheat foods made from red and white wheats, particularly where food and fuel shortages and poverty create a demand for nutritionally rich, inexpensive food that is partially processed to spare fuel. The adaptability of wheat to varied foods and its low price provide a basis for development of products for such markets.

Thirty percent of each grain of wheat leaves the mill as mill-run or separated mill streams, which is too substantial an amount to neglect. The optimum recovery of nutritious food or feed from mill-run is being sought.

Research is conducted by the Western Utilization Research and Development Division at Albany, California; under contract at Pullman, Washington; Lafayette, Indiana; Chicago, Illinois; Manhattan, Kansas; and under P.L. 480 research grants in England, France, Poland, Italy, Australia, Switzerland, and Israel.

The Federal program of research in this area totals 53.9 professional man-years, including one scientist whose salary is provided by the Farmers Co-Operative Commission Company under a Memorandum of Understanding and 10 contracts providing research at a rate of approximately 9.1 professional man-years per year. Of this number, 31.3 are assigned to investigations on chemical composition and physical properties; 21.4 on new and improved food products and processing technology; and 1.2 on new and improved feed products and processing technology. In addition, the Division sponsors 15 research grants under Public Law 480 including 13 on basic studies and 2 on applications of research.

PROGRAM OF STATE EXPERIMENT STATIONS

The State agricultural experiment stations conduct an extensive program of basic and applied research directed toward increasing or improving the utilization of cereals.

A number of these studies involve determining the influence of environmental, agronomic, harvesting and storage practices on the milling and baking characteristics of experimental lines as well as established varieties. Samples are analyzed for protein, moisture and ash content and are milled to determine flour yield. Physical dough properties and baking characteristics are evaluated to provide guidance to breeding programs and full knowledge of

quality to the milling industry. Testing frequently begins with laboratory micro-quality tests and extends through full-scale milling treatment. Examples of the program include study of gluten content, air classification and baking properties of high protein lines of wheat.

Newer types of malting barley and other barley having variable biochemical and enzymatic composition are studied in relation to harvesting, storage and malting quality. Basic constituents are determined and related to chemical and physical properties and used to assess commercial malting quality.

Several very basic studies designed to elucidate fundamental principles involved in conversion of cereals into food products are in progress. Determinations of the micromeasurements of physical grain properties are used to relate properties of the wheat and wheat products to flour milling technology. Investigation of the physical properties of small particles helps to understand bulk properties, the nature of the water-soluble gluten fractions, and in turn, the baking properties of the flour. Study of enzyme systems involves isolation of the enzymes and investigation of their substrate conditions, mode of action, specificity and application, for example, in malting. Detailed research on the amino acid content of wheat, especially lysine, provides information of interest from a nutrition standpoint. Study of the role of the lipids of durum wheat is providing insight into the role they play in determining the physical properties of alimentary pastes. Wheat protein fractions are being carefully characterized both chemically and physically. Investigations on the desulfurization of wheat gluten proteins and the changes in solubilities of gluten, gliadin and glutenin are being conducted. Hydrogen bonding in proteins is being studied to determine its role in determining the baking quality of wheat proteins.

The aroma and flavor of fresh bread and bakery products have universal appeal. Researches are in progress to determine the constituents responsible for the odor and flavor and how to retain them. Development of flavor and aroma through use of pre-ferments is also being investigated.

Attempts to develop new and improved food processing techniques or products are directed to: determining the physical structure and properties of doughs and the effect of freezing upon them; and to establishing better methods of wheat conditioning. Other work involves developing an understanding of the fundamental concepts involved in mixing dry solids and development of methods for producing water dispersible dry protein preparations from wheat. Stability tests and related investigations on a wheat wafer for shelter rations are being conducted in cooperation with USDA (WU, ARS).

Related research involves study of the economic feasibility of using barley and wheats of different quality and the supply and flow patterns of wheat.

The total research effort on wheat and barley utilization research is approximately 12.5 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Protein Interactions. The bread-making quality of wheat depends upon its protein. Good bread requires transformation of a flour paste into a strong, smooth dough by mixing and kneading. The amount and kind of protein available determines whether or not the dough will be elastic enough to retain its gas bubbles as the yeast works and at the same time be fluid enough to allow the gas bubbles to expand. The dough must be uniform so that the bread crumb will be even and attractive. As ancient as is the art of bread making, we are only now beginning to understand the fundamental chemical and physical actions involved. As dough is worked, chemical and physical bonds are made and broken between wheat molecules (intermolecular bonds) and end groups within molecules (intramolecular bonds). Non-protein wheat constituents and other ingredients (such as milk, fats, etc.) enter into the making and breaking of inter- and intramolecular bonds. The rate and extent of these reactions determine the quality, shape, and size of the baked loaf.

As in all natural systems, there are many dissimilar proteins in wheat. The study of their individual contributions to bread quality must begin with their separation and characterization. Chromatography and electrophoresis separate protein extracts made in the laboratory from wheat. Proteins can be separated by size, shape, and electrical charge of the molecules. Solubility, rate of movement on separation media, immune reactions, and enzymic activity can characterize the separated proteins. Solvent systems, packing of chromatographic columns, and gels and gel concentrations for electrophoretic separations are being developed and evaluated to separate and describe wheat proteins. Immunoelectrophoresis techniques have shown relative purity of the isolated fractions separated by column chromatography. Six fractions are obtained from the gliadin protein of wheat gluten. Further purification of these fractions is necessary for functional tests of individual proteins. So far attempts to fractionate the glutenin proteins of wheat gluten have been less successful and research will continue.

We reported before that as doughs are mixed, the solubility of proteins increases and the number of detectable sulfhydryl groups decreases. The rate and extent of the increase in solubility of proteins can be influenced by chemical blocking of sulfhydryl groups to prevent their interacting to form intra- and intermolecular bonds. As purified protein isolates become available to manipulate the protein content of doughs, their specific effects on protein solubility, sulfhydryl content, and rheological character of doughs and the quality of baked bread should become clearer. Such understanding in turn should provide a more rational description of the bread-baking potential of wheats and lead to methods of controlling raw materials and processing.

The interaction of proteins in wheat flour with nonfat milk solids (NFMS) is under investigation. NFMS is added to improve nutrition and other bread qualities, but continuously mixed bread doughs will not tolerate as much NFMS as conventional doughs. Thus the trend to continuous-mix operations substantially decreased the markets for NFMS. Various flours tolerate different NFMS addition.

Our research shows a change in the electrophoretic pattern of separated proteins when NFMS and wheat flour are mixed. The $alpha_s$-casein band (from NFMS) disappeared but two new, rapidly moving bands appeared. Heat treatment of the wheat protein extract before mixing prevented the presumed enzymic reaction and left the $alpha_s$-casein intact. Mixing NFMS with wheat flour also reduced the intensity of the wheat gliadin bands. The NFMS apparently reduced solubility of the gliadin, a result that would be expected to affect baking quality. Experimental results further showed that NFMS forms disulfide bonds with wheat proteins. Enzymic alteration of the $alpha_s$-casein may increase the opportunity for this type of bonding. If so, the $alpha_s$-casein splitting strength of wheat flour could be the key to toleration of NFMS in bread formulas.

2. Dough Rheology. Basic investigations supported by a Public Law 480 grant at the Rheological Laboratory of the Israel Institute of Technology at Haifa and at the Bread Research Institute of Australia in North Ryde, New South Wales, are relating flow characteristics (rheology) to the properties of wheat flour dough. These investigations seek fundamental data that will be useful in developing equipment and procedures for the testing of flour for bread-making quality. Present testing equipment, such as the Farinograph and the Extensograph, is useful but does not provide unequivocal predictions of baking quality. The project in Israel was initiated to test cylindrical dough specimens for tensile stress-and-strain relationships, relaxation time, and elastic recovery behavior. Data so obtained are to be compared to data on the same dough taken from the three characteristic stages of the Farinograph cycle. The dynamic nature of the system measured with the Farinograph caused difficulties. To remove samples from the Farinograph bowl, mixing had to stop. Stoppage rapidly altered rheological parameters. The alteration made the strain measurements unrealistic with respect to continuously worked dough. Preliminary measurements of the viscosity-stress relation indicate that a correlation may be feasible over a narrow range of conditions.

The new project just activated in Australia will supplement information obtained in Israel by different apparatus and techniques. For example, they will measure deformation of dough under a dynamic condition that should produce data on stress-strain relationships involved in dough mixing.

3. Analysis of Wheat Proteins. A way of estimating wheat flour proteins quantitatively was developed in contract work recently terminated at Washington State University. They extracted proteins from flour with aluminum lactate-lactic acid buffer and separated them by gel electrophoresis.

They then determined the amount of separated proteins by densitometric methods which depend upon the relative amount of protein and the darkness of the bands on the electrophoretic separation pattern.

The Washington State method was tested on flour of various types. Durum flour differed markedly from hard wheat bread flour. Durum was low in the slow moving proteins which correspond to the gliadin group. A club wheat sample had a protein distribution qualitatively similar to a hard red winter wheat sample of good baking quality but differed in the amount of several of the major gliadin protein fractions. Major quantitative differences in the gliadin band region and lesser differences in the albuminoid protein band region were found between air classified fractions of flour. Their new procedure should be useful in studying the relationships between flour composition and functional properties for baking.

Supported by another contract the same group at Washington State University is investigating the role of individual proteins in dough mixing by radioactive tags on wheat components. Flours labeled with radioactive carbon (C^{14}) were obtained from a hard red spring and soft spring wheat grown in the presence of carbon dioxide which contained C^{14}. They further labeled doughs by introducing amino acids and protein hydrolysates that contained C^{14} into the early dough stage of mixing. Electrophoretic separations of dough proteins will indicate interactions and changes. The C^{14} can be detected and can be used to measure protein changes. Such procedures, if successful, will be most useful in basic and applied studies of wheat.

Other advances were made toward useful analytical tools for wheat protein research. Pioneering research on immunochemical techniques is continuing under P.L. 480 at the Pasteur Institute in Paris, France. Two characteristics of protein are being juxtaposed to identify protein differences. Electrophoretic mobility and antigenic specificity of protein give complementary analytical separations and identifications of protein components. Preliminary separations by differential solubility of proteins in water or in ammonium sulfate solutions, by column chromatography with ion exchange adsorbance, and by Sephadex filtration, provide narrow groups of proteins to be analyzed by the immunochemical techniques. The refinement of measurement of protein components and changes of proteins during dough development, improve understanding of dough behavior.

The use of copper ion solutions for total protein extraction from various wheat flours is under investigation at the Cereals Research Station, St. Albans, England, supported by P.L. 480 funds. The extraction procedure using a solution of cupric and sulfite ions was applied to a range of wheat flours of different protein content and that exhibit different baking characteristics. The isolated protein contains up to 12% carbohydrate and is in the form of a soluble copper complex. The solubilization of protein did not appear to be connected with deamidation (a chemical process that will also increase solubility of glutenin). No evidence has yet been

obtained to indicate that the procedure significantly modifies amino acid residues other than cysteine-cystine. Extracts are being fractionated by precipitation behavior with varying pH and by column chromatography.

Chemical descriptions of the important flour protein system known as gluten have not been adequate for research because of uncertainty whether rigorous extraction and separation altered the native protein before it could be investigated. Mild ultrasonic vibrations are being applied to dilute gluten suspensions to provide residual material that may yield information on gluten composition. This research is conducted under P.L. 480 at the Cereal Biochemistry and Physical Chemistry Laboratories in Paris. The ultrasonic treatment slightly changed the viscosity and molecular weight, indicating some degradation of the gluten structure even by this mild action.

A basic investigation of nitrogenous components of wheat germ is supported by P.L. 480 funds at the University of Bologna in Italy. As reported before, significant amounts of polyamines were found in wheat germ. Research has been directed towards studying the nucleic acids, their purine and pyrimidine bases, and acid-soluble free nucleotides. These components are physiologically significant to life and growth, to the nutritional and, presumably, to the functional characteristics of wheat proteins.

Protein molecular size is studied in-house by measuring the migration rate of complex protein mixtures during electrophoresis. The concentration of starch in a starch gel for electrophoretic movement of proteins affects the migration rate of proteins in a way dependent upon molecular size. Additionally, some buffer systems that are used in electrophoretic separations affect protein mobilities by unfolding polypeptide chains. Preliminary studies in which gel strength was altered provided useful information about the soluble proteins of wheat. Further work is contemplated to specify analytical conditions that will yield more data to better describe protein composition.

4. Chemical Basis for Cohesiveness in Gluten. Basic research on the properties of gluten which contribute to the usefulness of wheat flours in food and industry, and to a greater understanding of how new applications may be devised, was concluded in contract work at the Midwest Research Institute in Kansas City, Missouri. A procedure was developed for preparing freeze-dried crude gluten, purified gluten and a glutenin component in quantities sufficient for detailed chemical and physical studies. The viscoelastic properties of crude and purified gluten and glutenin were studied as they are affected by water, pH, other components known to be present in bread doughs, and also chemicals that affect certain properties of proteins. Tensile strength and stress-relaxation determinations were made of gluten, glutenin, and preparations of these substances after chemical modification. Modification of protein sulfhydryl and amino groups affected the viscoelastic properties to a greater extent than other factors that were investigated. Data obtained from these studies provide a stronger foundation for future

basic research aimed at improving the utilization of wheat. Exploratory studies using the electron microscope were also conducted that provide interesting and apparently significant leads for further studies of chemically modified gluten and gluten preparations.

5. Enzymes in Wheat and Flour. Enzymes are proteins which in nature function to cause certain chemical changes. Flour proteins, yeast, and other bread ingredients are rich sources of enzymes. During mixing and fermentation, enzymes become increasingly active and cause chemical breakdowns and recombinations that affect bread quality. Studies are continuing to isolate and characterize amylase, proteinase, and lipoxidase enzymes which occur in wheat and wheat flour.

Proteins were extracted and separated in the usual ways and enzymic actions of individual protein fractions compared with the enzymic action of whole wheat and flour. Such investigations clearly indicated the presence of two major types of proteinases in wheat flour which differed in their pH maxima and ease of denaturation. Further attempts will be made to separate proteolytic enzymes from wheat and flour. The possible role of sulfhydryl groups in maintaining enzyme activity will be further explored.

In connection with the development of new immunoelectrophoretic separation and characterization of proteins at the Pasteur Institute in Paris under P.L. 480 grant funds (see paragraph 3 above), attention was directed to amylolytic activity of isolated proteins from wheat and barley. Alpha and beta amylases from barley malt were isolated and their ability to split amylose and amylopectin starch fractions characterized. Immunological techniques showed that the alpha amylase in the malt was quite antigenic. They tried to locate the same protein in an unmalted barley extract. Several immunochemical techniques indicated that no such protein exists in the barley prior to malting. On the other hand, a beta amylase with the same antigenic specificities was found in both barley and malt extracts. Solubility and mobility during electrophoresis varied with the origin of the extracted protein.

It is well known that moisture content influences changes in stored grains such as initiation of germination, loss of viability, and loss of bread baking quality. It is also known that enzymatic activities vary with moisture content. We presume the enzymic activity is related to the physiological and functional changes that occur when grain is stored. Studies of the lipolytic enzymes of cereal grains, particularly in relation to water content during normal commercial storage, are being carried out under P.L. 480 at the National Institute of Agronomic Research in Paris. This investigation has centered on two main points: (1) the development of new analytical methods that will reliably measure the slight lipolytic activity in dry grain storage and (2) lipolytic activity of germinating wheat. New gas chromatographic apparatus should allow determination of lipolytic activity at very low rates.

Amylolytic and proteolytic enzymes in wheat flour and malted wheat are being studied under P.L. 480 at the University of Poznan in Poland. This investigation seeks basic information on the function of sulfhydryl groups in these enzymes. The addition of sulfhydryl-blocking reagents reduced activity of beta amylase and proteolytic enzymes from wheat. Analysis of wheat proteins and enzymes indicated that only the cysteine-cystine residue had been altered by the sulfhydryl blocking reagents. Over a three-month period, wheat lost substantial beta amylase activity.

Standardization of biometric procedures and separation processes for wheat constituents are continuing in another investigation under P.L. 480 at the College of Agriculture at Poznan, Poland. This investigation centers on the role of riboflavin as a coenzyme of wheat endosperm to provide basic information related to enzyme modification of wheat components that occur during the processing and baking of wheat products.

6. Flour Maturation. Addition of oxidizing agents such as bromate, iodate, or chlorine dioxide, or holding for maturation improves baking performance of hard red winter wheat flours, particularly. Holding for maturation is expensive and the use of chemical agents is forbidden in several countries which would otherwise import large quantities of surplus winter wheat. Research is conducted to study the chemistry of maturation and the reactions involved in using oxidizing agents with the objective of making winter wheat more valuable for export, particularly to the dollar markets of Western Europe. First attention in this research was directed toward lipid material, enzymes that alter the lipids, lipoproteins, and enzymes that affect protein. Hard red winter wheat differs in lipid composition from hard red spring wheat which requires less maturation or chemical to perform satisfactorily in baking bread. Known phospholipid materials were purified for general use as reference standards to identify fractional components from wheat flour lipids. Laboratory procedures to separate complex glyco- and sphingo-lipid materials into simpler fractions were advanced but need further work. The isolation of lipoprotein material was initiated. Mercaptans have been observed elsewhere to influence peroxide formation from fatty acids. The peroxides formed from unsaturated fatty acids can destroy sulfhydryl groups of proteins. Studies of such changes in wheat flour were initiated to isolate and investigate the various reaction products formed by lipid oxidation and the reaction of oxidized lipids with proteins.

It was reported earlier that as dough mixing proceeds the amount of protein that can be extracted with dilute acetic acid increases. Protein residues from such an extraction tend to be gelatinous. The gelatinous residue is being studied to characterize its proteins. The nitrogen terminal groups indicate the protein fraction of the gel is made up of very similar subunits. Glucose-containing polymers from the gel were separated by a mild procedure and the protein fraction was partially purified. Xylose residues from the separation indicate that a glycoprotein may be present.

Additional research is being conducted at Kansas State University to identify protein and lipid qualities that may alter response to oxidative maturing treatment of spring and winter wheat flours.

7. Lipids and Lipoproteins. Proteins can combine with lipids to change bread doughs and thus the quality of bread. The National School of Agricultural and Food Industries and the National Institute of Agronomic Research in Paris conducts research on the lipids, phospholipids, and lipoproteins under two P.L. 480 grants. In one project wheat and wheat fractions are analyzed for lipid and lipid-related components. The effects of removing lipid from flour on dough mixing strength were studied. They are trying to find out what lipids do and what happens to them during maturation of wheat flours. The second grant directs particular attention toward phosphorus-containing compounds and their relationship to lipid material. Less phosphorus in the form of phytic acid and its related salts is found in winter wheats, both soft and hard, than in hard spring wheats; durum wheat has substantially more phytic acid phosphorus than either. The phosphorus composition of wheat gluten in soft wheats is low in phytate and nucleic phosphorus, but high in inorganic phosphorus. The opposite is true for hard wheat glutens. Durum wheat samples were intermediate in organic phosphorus. These and similar findings are beginning to reveal specific compositional differences between wheat flours which differ widely in baking.

Albumin, globulin, gliadin, and glutenin proteins are also being characterized as to their content of specific phosphorus-containing compounds. Such studies broaden the foundation needed to build a better understanding of the relationship of composition of wheat flour to baking quality.

8. Bread Flavor. The flavor of freshly baked bread is one of mankind's delights. The instability of fresh bread flavor causes rejected slices and uneaten crusts. Basic research is aimed at revealing the chemistry associated with bread flavor. By understanding what flavor is and how to measure it, we start toward flavor enhancement and flavor stabilization. The ultimate objective is to produce bread that will maintain a fresh aroma instead of going stale.

As analytical techniques become progressively more sensitive and reliable, the chances of adequate understanding of the chemistry of bread flavor and aroma improve. Many bread components which exist in concentrations of fractions of a part per million were separated and identified. Bake oven aroma was captured and concentrated in a distillate. Several drops of the distillate on a hot surface at about 200° C. produce vapors that are reminiscent of the tantalizing odor of fresh baking bread. Distillate lasted for more than three months under refrigeration without substantial change. Preliminary chemical studies of the preserved bake aroma distillate correlated with informal subjective judgments. More critical experiments will require formal trained panels for subjective evaluations.

Specific chemicals have been used to treat the bake oven distillate in order to learn the chemical makeup of bread aroma. When reduction with sodium borohydride removed carbonyl compounds, the typical fresh baked bread aroma disappeared, but a strong yeasty odor remained. Oxidizing agents such as acid permanganate and aqueous bromine also destroyed the fresh bread aroma. Dilute alkali did not affect the odor, indicating that the aroma components are not acidic.

We have studied changes in concentration of certain alpha-keto acids during the fermentation of pre-ferment brews. Alpha-ketoglutaric acid and alpha-ketoisovaleric acid increased slightly during fermentation. Pyruvic acid, on the other hand, increased rather substantially over six hours of incubation.

Stability studies of food products made with expanded or puffed bulgur are conducted to determine the chemical nature of storage deterioration. Of particular interest has been the observation that hydrocarbons (ethane, propane, butane, and pentane) were products of deterioration. We found that when pure methyl linoleate was oxidized it also produced these hydrocarbons. They appear to be important in storage stability investigations because they can be detected objectively long before the first detectable oxidation in the product.

B. New and Improved Food Products and Processing Technology

1. Bulgur and Related Wheat Products. Bulgur, a parboiled dried wheat, known since ancient times in the Near East, has been in limited production for many years in the United States using traditional open pot cooking and sun drying or other inefficient drying methods. Early in the 1950's a collaborative program with participation of the U.S. Department of Agriculture's Western Regional Research Laboratory,the Oregon Wheat Commission and Wheat Growers League, the Millers National Federation, and Fisher Flouring Mills, Inc., began exploration of modern production methods and export sale of bulgur. In 1961, the Food for Peace Program included 60 million pounds of bulgur for welfare distribution in 27 foreign countries as a trial. The success of this trial was immediate and by 1964 these new markets for wheat, which resulted from utilization research and a cooperation of government and industry, reached 8 million bushels a year distribution with demand still growing. Part of the bulgur was produced by a pressure cooking method developed by Fisher Flouring Mills in the early stages of the cooperation and another part was produced by a continuous process at atmospheric pressure using technical information developed by the Department.

Investigations of bulgur are continuing. Puffing of bulgur by both explosion from a pressure gun and exposure to hot dry air are being evaluated. Expansion by explosion puffing was considerably greater than by hot air puffing. In explosion puffing the amount of pressure built up in the gun controlled the degree of expansion. The optimum moisture content of the grain for this process is about 19%. New equipment was installed for hot

air puffing to provide precise control of temperature and air flow conditions over the appropriate range. The equipment is directly related to existing commercial-scale continuous puffing equipment.

Rotating or tumbling pressure retorts have made it feasible to can bulgur by mixing dry grain with water in the can and cooking during the agitation. Formulas were modified for this method and a series of demonstration samples was prepared to indicate flexibility of bulgur as an ingredient for various menu items.

A variety of seasoning packets was developed that are specifically suited for use with dry bulgur and can be incorporated into convenience formulas based on instant bulgur. They provide dry, light-weight counterparts of many of the canned bulgur products.

Bulgur fines, resulting from the production of cracked bulgur, were used in the formula for two sweet bread products that can be preserved in hermetically sealed cans. Date nut bread and Boston brown bread made with bulgur fines are quite attractive products.

The wide variety of attractive and nutritional bulgur formulas developed earlier were modified for institutional-scale cooking. Demonstrations have been made before associations of wheat growers, professional food groups, and social organizations.

Peeling bran from wheat removes fiber and pigments but leaves the nutritious aleurone layer. Considerable success has been attained by peeling wheat with lye. Attractive peeled wheat has been produced in the laboratory and further attention will be given to vitamin retention and reduced cooking time so that the new product can be prepared with minimum inconvenience and fuel. Investigation of the storage stability of peeled wheat products is going on.

2. Food from Wheat Fractions. New high-protein food products from wheat are being investigated. Such products would utilize separated wheat gluten, high-protein flour fractions obtained by air-classification of finely ground flour, protein extracts and concentrates of wheat and flour, and protein concentrates and extracts from mill feed. A hard red spring wheat bran that had 22% protein was further milled and air classified to yield about 1/3 of the starting material as a flour-like powder that had 31% protein. The same bran was extracted with salt solutions, yielding a quarter or more of the starting material in the range of 29-38% protein.

New products from wheat gluten included gluten-based prototypes of beef stew and chicken drumsticks with better texture and flavor characteristics than possible in earlier work. Preliminary work indicated the feasibility of simulating Chinese sausage using gluten instead of pork and beef.

If wheat protein were more soluble it could be dispersed in water and made into a bland, milk-like, nutritious drink. Modifying amide end groups does

solubilize wheat protein, and research to accomplish this is under contract at Purdue University. Extraction of protein from wheat flour by dilute acid in combination with pepsin, a protein-splitting enzyme, is promising. The acidity of the extract made it possible to deamidate the protein by heat without further acid or salt. About 85% of the total protein from the flour was recovered in the extract with one washing. Variables in this operation, including pH, time, enzyme concentration, and ratio of flour to extractant, are yet to be worked out in detail. Studies with a glutenin preparation were conducted to provide guidance. Glutenin is the portion of the flour protein that is the most difficult to disperse. The principal problem is to deamidate and split insoluble proteins to improve their solubility, without freeing an excess of individual amino acids that would brown during heat processing and subsequent storage of dried or liquid products.

The development of powdered formulations from wheat fractions or extracts high in protein, minerals, and vitamins would be useful as a food supplement in the protein-short areas of the world, particularly for infants and preschool children.

3. Emergency Food Supply. Investigations of foods suitable for fallout shelters are being conducted with funds transferred to Agriculture by the Department of Defense. Close cooperation was given by Department scientists to the Office of Civil Defense and the Van Brode Milling Company of Massachusetts in commercialization of bulgur wheat shelter wafers developed by the Department of Agriculture. The Defense Department bought more than a million pounds in an initial order for stockpiling in fallout shelters. Subsequent orders have been larger.

Advances in formulation and processing the bulgur wheat wafer and evaluation of its stability characteristics continued. Research also continued on inexpensive stable food adjuncts for use with the bulgur wheat wafer to improve wafer acceptability by varying the form in which it is eaten.

Stability studies of bulgur wafers are continuing at Oregon State University, supported by contract funds. In addition, stability studies of several selected food adjuncts are also being conducted at Oregon State University under a second contract initiated during the past year. Variables in the bulgur wafer stability study include red and white wheat as the basic material, malt and corn syrup in the formula, nitrogen vs. air pack, and storage at 40, 70, and 100° F. The food adjuncts are spreads, soups, sauces, etc. and include raspberry jelly, strawberry spread, cream of chicken soup, beef soup, oriental sauce, curry sauce, paprika gravy, chili sauce, apple topping, butterscotch topping, chocolate pudding, and wild cherry icing. Samples for the stability tests are evaluated every six months by a taste panel using a 9-point hedonic scale. In addition, each sample is being evaluated at these intervals by an experienced taste panel of four persons judging color, flavor, odor, ease of preparation and rehydration, and evidence of deterioration. Samples are packed in tin cans, half with nitrogen

atmosphere and the other half with air pack. Storage temperatures are 40, 70, and 100° F.

New food adjuncts for wafer-based rations are being developed, including a dry product that can be instantly converted to a fruit-flavored jelly by mixing with cold water, and a margarine-type spread that has long shelf life.

Basic studies of the oxidative deterioration of wheat bulgur wafers are being conducted to develop accelerated stability tests that will predict slow changes in a short time.

4. Baking Quality. One method for determining the effects of individual components of flour on baking quality is to separate flour into constituent parts; purify individual components; and add them back individually or in closely related groups to see how they affect baking. By fine grinding and air classification, flour was divided into high-, intermediate-, and low-protein fractions. When high-protein fractions from hard red winter wheats were added to low-protein flours they increased mixing stability. Varietal differences were observed in the low-protein flours as well as the hard red winter wheats which were the source of high-protein fractions. A third re-grinding of the high-protein fraction decreased mixing stability.

Gluten, which was separated from flour, was combined with a low-protein flour fraction. Crude gluten blends gave dough mixing curves that were not substantially different from doughs made with the low-protein fraction without gluten addition. This also indicated that some material was lost or the state of organization of the gluten was changed by the methods used to recover the gluten, so that its original contribution to baking quality was lost. Soluble protein fractions are being isolated for further tests of the relationship of individual components to baking quality. We expect fractionation will not alter the more soluble proteins as much as it does the gluten and that they may still retain their original function in bread doughs.

Air-classified wheats were also used to bake cookies. Varietal differences again appeared. Varieties with poor bread baking strength were not necessarily good for cookies. Triumph variety was not as strong as Comanche and Bison for bread strength but yielded low-protein air-classified fractions that were superior for cookies to Pawnee, which was the weakest of four varieties tested.

The effect upon baking of variation in wheat flour lipids is being investigated under P.L. 480 at the British Baking Industries Research Association in Chorleywood, England. The second year of a survey of five United States bread wheats and, for comparison, two European varieties has been completed. Oxidative change of lipids is believed to be involved with the change of sulfhydryl to disulfide links in protein of bread doughs which affects dough strength and mixing stability. The first year survey indicated that baking quality varied with lipid composition, but the specific relationship did not hold the second year. Varietal differences, however, were observed in both

surveys. Lipids added to bread formulas improved baking quality and oxidation is thought to be involved. Time lapse motion pictures were used to observe and measure the effects of adding lipid material to doughs. When lipids were added, the dough rose faster in the bake oven and set later. The lipid appeared to affect the plasticity of bread doughs, making for a bigger loaf volume and more uniform textured crumb.

5. Nutritive Value of Processed Wheat. Heat and chemicals applied during the manufacture of bulgur and other wheat products may harm nutrients. Wheat nutrients are especially valuable in some overseas markets where wheat products are being used to overcome specific nutritional deficiencies. Studies to develop rapid chemical methods for assay of nutritive value of wheat proteins during processing are being conducted under P.L. 480 at Cambridge University in England. Compositional and biological measurements were made of the effect of heat on certain essential amino acids in wheat products. Comparisons were made of uncooked wheat and three bulgur samples cooked by different processes. The first process was equivalent to commercially prepared bulgur. The other two processes were successively more severe in their heat treatment. Biological protein values, established by rat-feeding tests, indicate no loss of protein nutrients caused by the bulgur process. The two over-processed bulgur samples lost 10% in biological value. Chemical analyses of individual amino acids were conducted to seek correlations with rat- and chick-feeding tests.

In-house developments of new peeled wheat products include analysis of vitamin retention during processing. Lye peeling of wheat reduced thiamine and riboflavin nearly 50%. Attempts to translocate thiamine by soaking prior to the lye peeling operation have not yet been successful. Research to protect the B-vitamins in peeled wheat will continue. Rat feeding tests indicated that parboiling and drying wheat in bulgur manufacture do not reduce the protein values. However, the heat-puffing of bulgur, in order to make it more convenient for rehydration, lowered the protein value significantly.

6. Elimination of Microbial Contamination of Wheat Flour. The safety of many foods depends upon their relative freedom from microbial contamination. Starting ingredients may contaminate prepared foods. Elimination of microbial contamination from flour would make it more desirable for many uses including precooked frozen foods, baby foods, and certain canned products. Research is being conducted under contract by the American Institute of Baking in Chicago to determine the nature and extent of microbial contamination in wheat flour and means for reducing or eliminating it. Three species of bacteria and one mold were selected as test organisms to represent major species likely to be natural contaminants in commercial flour. Research procedures were advanced to inoculate flours with pure cultures of bacteria and molds and to count the numbers of organisms remaining viable in flour. Bacteria die quickly on dry flour. E. coli and staphylococci decreased by

more than half in five days at room temperature. Such background information will provide a base line for evaluation of methods for eliminating bacteria from flour.

C. New and Improved Feeds and Feed Processing Technology

1. Improved Feeds from Wheat and Wheat Fractions. Research on feed utilization of wheat and wheat fractions is continuing at a low level of activity, limited by unavailability of personnel. Protein Efficiency Ratios (P.E.R.) of wheat following various treatments were obtained by chick feeding tests. While these studies were primarily concerned with effects of food processing variables on wheat nutrients, they provide background knowledge for the feed utilization of wheat. For example, moist heat treatment of wheat (as in parboiling for bulgur manufacture) was found to have no adverse effect on lysine availability. In addition, bulgur had a greater growth promoting effect than the wheat it was made from when maximum lysine was added. Lysine availability and P.E.R. tests carried out with chicks closely correlated to those with rats. It is planned to extend the investigations on P.E.R. and lysine availability to mill byproducts and fractions obtained from them. Cooperative work was initiated with two mills in the Pacific Northwest.

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AREA NO. 2. RICE--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The productive capacity of U.S. rice growers has increased faster than domestic and export consumption over the past decade, limiting the income potential of this major world food grain. Detailed knowledge of chemical composition and physical properties, as related to processing, is needed to guide milling, processing, and product development of U.S. rices so that they can better meet the quality requirements of expanded markets. New and diverse food products from rice that are easy to prepare, have flavor and texture appeal, and are economical to manufacture, are needed to increase the total consumption of rice both here and abroad.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on rice is conducted at the Division headquarters, the Western Regional Research Laboratory in Albany, California. Basic studies involve chemical, physical, and biochemical investigations of rice proteins and of processing. The protein work is concerned specifically with isolation and characterization of the globulin and glutelin proteins of the endosperm. Effects of processing on quality of cooked rice are being studied. Preparation of high-protein rice fractions by means of fine-grinding and air-classification is under exploratory investigation.

The Federal program of research in this area totals 3.1 professional man-years. Of this number 1.9 are assigned to chemical composition and physical properties; 1.2 to new and improved food products and processing technology.

PROGRAM OF STATE EXPERIMENT STATIONS

The program on rice involves evaluation of new rice varieties and lines for specific uses through cooperation with the Regional Rice Quality Laboratory. Early emphasis is placed upon developing and applying rapid and simple testing procedures for screening the selections. Subsequently, the influence of cultural methods, drying procedures and storage upon processing and product quality is determined. Basic compositional and other data relative to the quantity and quality of the proteins, lipid and starch as well as methods of parboiling are obtained.

The effectiveness of infrared drying of rough rice, as measured by rapidity of drying and maintenance of melting quality, is being studied. Results to date indicate that infrared-dried rice may deteriorate less rapidly in storage. This study will be expanded to include other varieties and the effect of maturity at harvest.

Fundamental studies on the chemistry of rice are directed toward study of the variation in the constituents of several varieties. Both chemical and physical properties are observed and related to differences in quality of rice.

New product research centers around development of effective ways to use rice in quantity food service. Products are developed and evaluated for acceptability, ease of preparation and costs.

Extended utilization of rice will be facilitated by better characterization and utilization of its proteins. This is especially true in the underdeveloped countries where rice is the principal food. Continued study of the supplementary value of high protein foods for rice and its by-products and of rice proteins for those of corn and wheat is in progress. The amino acid composition of rice is being determined since it may vary with variety and other environmental factors. The biological value of the proteins of rice when used with multipurpose food is also being investigated.

The total State scientific effort devoted to utilization of rice is 1.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Rice Proteins. Investigations of processing, cooking, and nutrition of rice invariably lead to unknowns about rice proteins. Rice normally contains too little protein to sustain healthy human life by itself. Although the quantity is low, the quality is high. Rice protein has an especially high biological value for a cereal source protein. Therefore, basic research on protein constituents is being conducted. Rice protein was extracted and separated by solubility differences into globulin, prolamine, and glutelin fractions, which are being further separated and characterized. Principal attention was directed to the globulin fraction, reported before to contain more than 11 components as detected by electrophoretic separation. Rice globulin was separated into insoluble and soluble fractions by changing acidity and ionic strength of extracting solutions. The soluble fraction, which contained an unusually high level of sulfur for a cereal protein, was shown by electrophoresis to contain one main and several minor components. Progressive heat coagulation under increasing temperature conditions further divided the globulin fraction. About three-quarters of the material was coagulated at 80° C. Analysis of the coagulated material indicated that the high sulfur fraction remained uncoagulated at this temperature. The usefulness of this technique is limited because the heat-coagulated material is too insoluble for further characterization by electrophoresis.

B. New and Improved Food Products and Processing Technology

1. Improved Rice Products. For a major part of the world's population, rice is the principal source of calories. Improvement in nutritional quality of rice would improve the nutritional status of hundreds of millions of people. High-protein rice flours were produced by turbomilling and air classifying rice flour. Starting with rice grown at the California State Experiment Station with heavy application of nitrogen fertilizer to increase protein content, rice flour fractions ranging from 11-17% protein were produced by turbomilling and air classification.

Others have reported that the rice endosperm is richer in protein at the surface than in the center. A milling procedure that peels away only the outer portions of the endosperm was successful. Commercial separation of high protein flour from rice appears possible because conventional equipment can be used and the partially milled grains can be sold in regular channels.

California exports much of its rice to the Caribbean area where high-gloss polished rice is preferred. The high gloss is achieved by polishing with talc, which cannot be metabolized. Regulatory agencies have questioned talc application. The substitution of certain calcium salts, which can be metabolized, for talc has shown promise. Calcium citrate produces a sheen almost like talc with no apparent change in odor or flavor. Testing of other materials will continue.

Vitamin deficiency diseases remain prevalent in many areas of the world that import American-grown rice. The enrichment of rice in a rinse-resistant coating would improve nutrition from rice. Current practice is to enrich a small number of grains at a very high level and then mix them in with untreated rice. If all the rice is to be treated, the coating that contains the added nutrients must be able to resist the rinsing and washing that may occur before the rice is cooked. The inclination of rice to check and crack if it is moistened makes application of nutrients more difficult. In preliminary investigations two approaches appeared promising. Ingredients for enrichment were applied to the rice kernels in a dispersion of adhesive components insoluble in cold water. Alternately, application was in several steps with coating materials of low solubility or materials that could be made insoluble after they are applied to the kernels.

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AREA NO. 3. FORAGES AND FEED-- PROCESSING AND PRODUCTS

Problem. The demand for livestock in the United States will increase 45% by 1975. Forage crops constitute the major feedstuff for ruminant animals. In addition, there is an increasing demand for processed forages in European and Asiatic export markets. Fresh forage crops are the richest natural source of many nutrients for farm animals. The bulk of forages, however, is preserved so inefficiently by haymaking and ensiling that 10 to 50% of the dry weight, and much larger fractions of the most valuable nutrients, are lost before the animals eat them. Dehydration is now the only practical means of producing high nutritional value products in a form usable in manufactured feeds and supplements. Poultry and swine producers are aware of the value of dehydrated forage, but restrict their consumption because of high fiber and growth-inhibitor content. The livestock breeder needs forage products tailored to specific animals, and the forage producer must adapt to his needs to sell.

Basic and applied utilization research are necessary to produce: (1) high protein, low-fiber feeds rich in unidentified growth factors designed for use by non-ruminant animals; (2) fiber products which have been cheaply treated to make them easily digestible for ruminants; (3) growth stimulating supplements for ruminants based on the biologically active fiber digestion factors and growth-promoting factors in forage. New products should be adaptable to mechanical feeding. Improved uses will encourage farmers to put high-value land now producing surplus crops into forages.

USDA AND COOPERATIVE PROGRAM

Current research in the Western Utilization Research and Development Division includes both basic and applied studies on all forages used or potentially usable for off-the-farm processing. The research is conducted at the Division headquarters at Albany, California; under contract at Berkeley, California and Athens, Georgia; and under the P.L. 480 grant programs in Edinburgh, Scotland, Lodi, Italy, and Helsinki, Finland. Basic compositional studies deal with the potent estrogen, coumestrol (discovered by Department scientists), and other phenolic compounds present in forage legumes. The value of coumestrol-rich alfalfa as a growth stimulant for ruminants is being studied with financial support of the American Dehydrators Association and the cooperation of ARS Farm Research and several experiment stations. Also under study are other biologically active forage constituents (such as the chick growth-promoting factor in forage juices and alfalfa saponins which depress chick growth), organic acids of alfalfa, and the mechanism of action of forage antioxidants. Processing of forages by "wet" (juicing) and "dry" (turbomilling and air classification) methods is being investigated.

The Federal program of research in this area totals 13.4 professional man-years, including one scientist whose salary is provided by the Department of Agriculture and Inspection, State of Nebraska, and contract research equivalent to 1.9 professional man-years per year. Of this number 6.2 are assigned to chemical composition and physical properties; and 7.2 to new and improved feeds and processing technology. In addition the Division sponsors, under P.L. 480, three research projects on forage composition. The American Dehydrators Association has provided funds to cover the salary of a 1964 summer employee for study of the cellulosic constituents of dehydrated alfalfa.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct an extensive program of both basic and applied research on forage utilization. Much of the research is interdisciplinary and often involves several departments.

One major segment of the research effort is devoted to determining the chemical composition of forages and evaluation of the relationship between chemical composition of certain forages and their nutritive value for farm animals. Evaluation of the effects of certain agronomic, cultural, processing and handling practices on composition, palatability and nutritive value of forages receives much research attention. Fiber content and utilization of fiber by swine, cattle, sheep and poultry affect the value and use of forages. Methods of isolating and analyzing for fiber are being developed. Investigation of normal and abnormal rumen fermentations of forages is fundamental to maximum utilization.

Careful studies of specific constituents of forages are being undertaken. Determination of certain minor elements found in forages is important both from nutrition and toxicity standpoints. Protein content and quality merit special attention along with determination of amino acid values and unknown growth factors. Leaf organic acids and proteins are investigated in detail in an effort to increase our understanding of their biosynthesis and properties in relation to growth of forage plants.

Due to the economic importance of forages in animal feeds, development of means for evaluation of the nutritive quality of forages has become an important field of study. New and more accurate or rapid chemical procedures are being sought.

Development of forage handling and processing systems to minimize labor costs has led to increased research on forage processing methods. Fermentation characteristics of and animal response to forages which have been wilted, chopped, pelleted, ensiled or dehydrated are being determined. Small scale ensiling systems are being used to evaluate various silage preservatives. Methods of dehydrating alfalfa are being studied and the economic feasibility of dehydration is being investigated.

The total research effort devoted to forage utilization is about 17.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Chemical Constituents of Forages. Compounds that exhibit biological activity were extracted from alfalfa and ladino clover. Phenolic compounds were isolated and characterized from alfalfa and ladino clover and will be further tested for biological activity by the Pharmacology Laboratory and cooperating researchers in universities and commercial laboratories.

2. Estrogens from Forage Crops. Coumestrol is a compound in alfalfa and other forage crops that has estrogenic activity. The activity is measured biologically by an increase in the weight of immature mouse uterus from animals that have been fed the compound or feed containing the compound. Coumestrol has also been produced synthetically from flavylium salts, in connection with basic research on fruit anthocyanin pigments. The synthetic coumestrol and coumestrol extracted from alfalfa were shown to have identical biological activity. The availability of synthetic coumestrol made possible more extensive animal assay than was possible using the extracted material. Coumestrol in the diet produced temporary sterility in male mice with one gram of coumestrol per kilo of diet. In subsequent experiments, however, the level of coumestrol required to inhibit spermatogenesis in the male mouse was well above the level required to inhibit reproduction in the female.

3. Interaction of Forage Antioxidants. Contract research was initiated at the University of California, Berkeley, on a basic investigation of relationships of lipid to carotene oxidation and the bearing these compounds may have on other chemical constituents of forage crops. Thin-layer chromatography provided a means of rapid separation of lipids to monitor preparative column separation of these compounds. Forages have been found to be rich in galactolipids. Linolenic acid constitutes as high as 80% of the fatty acid content of certain fractions. Rates of oxidative deterioration of fatty acids and carotene were determined in alfalfa stored at relatively high temperature. Carotene destruction by oxidation was over 10 times as rapid as linolenic acid destruction.

4. Structure of Alfalfa Polysaccharides. The digestibility of alfalfa polysaccharides is apparently reduced by chemical combinations with undigestible lignin components. As a basis for improving digestibility of alfalfa products a structural analysis of alfalfa polysaccharides is being conducted under P.L. 480 funds at Edinburgh University in Scotland. They are following two lines of research. Improved extraction procedures are being developed to obtain individual polysaccharide fractions for further identification and study and to survey carbohydrase enzyme activity of extracts of dormant and germinating alfalfa seeds, stems, and leaves. Extraction

schemes were modified and developed to isolate polysaccharides from combined leaves and stems and also carried out on bulk quantities of separated leaf and stem fractions. Preparation of pure xylan hemicellulose samples is almost complete and these will be examined in detail in current work. Carbohydrases of alfalfa were found in highest quantity and most easily separated from alfalfa seedlings. Continuous electrophoresis separated proteins having different enzymatic specificity. Specific carbohydrases include alpha- and beta-glucosidases which are differentiated by the types of linkages that they break in disrupting the complicated carbohydrate polymers in alfalfa.

5. Organic Acids of Alfalfa. Continuing research on organic acids of alfalfa has shown that methodology is still inadequate for complete resolution of the complex mixture present. Current results show that a gradient elution column chromatographic method has greater promise than procedures used previously.

6. Natural Antioxidants of Alfalfa. P.L. 480 research in Italy is under way on isolation of non-tocopherol, ferric chloride-reducing lipids of alfalfa. No reports have been received thus far.

B. New and Improved Feeds and Processing Technologies

1. Coumestrol-Enriched Feeds. Encouraging results from sheep feeding with coumestrol-rich extracts led to preparation of substantial quantities of pure crystalline coumestrol. Samples were supplied to the Oregon State Experiment Station and to the Animal Husbandry Research Division of ARS for evaluation of coumestrol as a growth factor in sheep and cattle. Additional material was prepared to supply the Indiana State Experiment Station for further studies on steers. Initial experiments with steers at Indiana State Experiment Station showed no growth response with either high- or low-coumestrol meal or with meal fortified with coumestrol concentrates. However, in the most recent experiment 12 steers on alfalfa fortified with 100 p.p.m. of crystalline coumestrol produced an average 8% greater gain than appeared in control steers after 68 days of feeding. Further testing of the effects of crystalline coumestrol on weight gain of cattle will use identical twin calves.

2. Improved Alfalfa Meal. Substantial quantities of water-soluble saponin and soya-type water-insoluble saponin were isolated from alfalfa for chick feeding experiments. The soluble saponin inhibited growth but the insoluble type did not. The inhibition, however, was small and does not seem to be of practical importance in poultry feeding.

Studies on the improvement of alfalfa products by dry fractionation are being conducted, supported in part by the Nebraska Department of Agriculture. Several screening devices were tested and improved. Samples of alfalfa from

Kansas, Nebraska, and California, representing four varieties, were studied. Protein content of leaf fractions has been upgraded on the average of 7% above whole alfalfa meal and the fiber content of the leaf fraction was lowered by 10%. Carotene increased nearly 60% in the leaf fraction. Plans for a field-scale study of dry fractionation of dehydrated alfalfa were advanced and a commercial dryer will cooperate in the study in the summer of 1964.

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New and Improved Feeds and Processing Technology

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1/ Research supported by P.L. 480 funds.

AREA NO. 4. WOOL AND MOHAIR-- PROCESSING AND PRODUCTS

Problem. Traditional markets for wool and mohair have been lost to synthetic fibers because consumers prefer garments that hold their pleats and creases, resist shrinkage and wrinkling during washing, and dry quickly. Natural wool and mohair outclass the synthetics in tailorability, comfort in wear, appearance, and hand, but demand certain features now being exploited by the promoters of synthetics. Furthermore, some current processing damages, distorts, or weakens wool and mohair fibers and injures performance and appearance of fabric. We need processes that will modify natural fibers to give a range of comfortable and attractive fabrics that resist deterioration in processing and wear. Fabrics must be durably resistant to wear, wrinkling, pilling, abrasion, yellowing, soiling, felting and relaxation shrinkage, acid and alkali weakening, insects, and microorganisms. New markets would develop for new types of fabrics, woven and non-woven, for industrial and other uses, made with natural wools and with blends of wool with modified wools or other fibers. Wool could have a part of the new, rapidly developing market for stretch fabrics if we could practicably impart permanent stretch into wool yarn. Research toward such developments requires fundamental information on the chemical, physical, and structural nature of natural fibers and their modified products.

To sustain a stable sheep and wool industry in the United States, mills must be supplied with processing information on new and improved wool and mohair products. Synthetics have cut into wool markets because they are uniform in price and quality and because detailed processing information is available from producers.

USDA AND COOPERATIVE PROGRAM

The Western Utilization Research and Development Division conducts a broad basic and applied research program on wool and mohair to develop new and improved fibers and fabrics that can increase markets. Fundamental research seeks new facts on chemical and physical properties of natural fibers that may make wool and mohair fabrics more useful and valuable. We use such knowledge to try to modify fibers and fabrics so that they will resist degradation by heat, light, chemicals, staining, abrasion, and insects; wash easily; retain creases; shed wrinkles; and require little care. We seek practical processes for chemical and physical modification of wool and mohair fibers, yarns, fabrics, and felts into products that will increase wool and mohair utilization. In addition, Department scientists make every possible effort to bring research results to the industry through technical publications, public service patents, popular articles, TV and radio broadcasts, participation in growers' and processors' meetings, exhibits, mill visits and development trials, and conferences with visitors from the industry.

The Federal program is conducted at the Division headquarters at Albany, California by contract in Durham, North Carolina and Washington, D. C., and by grant funds under P.L. 480 in India, France, Sweden, England, and Finland.

The Federal program of research in this area totals 32.9 professional man-years, including contract research equivalent to approximately 2.0 professional man-years per year. Of this number 11.2 are assigned to chemical composition and physical properties and 21.7 to new and improved textile products and processing technology. In addition, the Division sponsors research grants under Public Law 480 including five on basic studies and two on the application of research findings.

PROGRAM OF STATE EXPERIMENT STATIONS

Station research related to wool and mohair utilization consists of one study designed to determine the comparative resistance to outdoor weathering of four wool fabrics made from fibers differing in crimp and fineness. A second study deals with the relationship of fiber quality and measurement to wool marketing practices. A third study is directed to providing information on the characteristics and properties of Texas mohair, including determining domestic and foreign attitudes toward utilization of mohair.

The total research effort devoted to wool and mohair utilization research is approximately 0.6 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Chemical and Molecular Properties. Basic physical and chemical investigations continue for the purpose of providing a rational basis for modifying and processing wools and wool fabrics into new and superior products. These studies undertake to locate reaction sites for alteration of fiber properties and to identify specific and type changes in fibers as they are subjected to radiant energy, mechanical manipulation, and chemical reactions.

A method to divide or break up wool fiber into component parts with a minimum of side effects would implement research. Grinding wool fibers in a ball mill at -196° C. fragments the fiber without chemical alteration. Fiber fragments so obtained can be separated by differences in density. A heavy fraction was obtained containing more sulfur, cystine, and proline than the lighter fraction. The lighter fraction is richer in tyrosine and phenylalanine.

Wool protein molecules may also be analyzed by enzyme digestion. Proteinases were used to digest normal wool fibers and fibers that had been coated with synthetic polymers. Partial treatment yielded protein molecule fragments for further study. Protein digestion of coated wool (wool treated with a surface coating or synthetic organic compounds) removed the wool but left

the surface film in a suitable condition for microscopic and electron microscopic studies. The objective of these investigations of surface films is to find means to strengthen surface coatings, while reducing the amount of material in the coatings.

Mild pepsin treatment of wool fibers showed the presence of an enzyme-resistant core in studies supported at the University of Lille in France by P.L. 480 funds. At Lille they separated wool protein into two fractions chromatographically. The major fraction corresponded to alpha-keratins with high molecular weight. Another fraction, 5% or 10% of the treated protein, was very high in glycine but low in sulfur. It was insoluble at pH = 3.9 and is now under further study.

Structural studies clarified a discrepancy between calculated and observed nuclear magnetic resonance data for glycine, a major component of wool by identifying differences between alpha and gamma forms of the amino acid molecule. Paramagnetic resonance absorption curves of glycine and deuterated glycine (that is, glycine in which heavy hydrogen has replaced an atom of normal hydrogen in each molecule) checked the positions assigned to the hydrogens in the glycine molecule.

Isocyanate treatment of wool fibers makes possible a graft between molecules which stabilizes the fiber structure. It is possible to promote intermolecular grafting inside the fiber after treatments which swell the fiber. In the absence of the swelling agents, the grafting is largely confined to the fiber surface. Isocyanate treatment imparts shrink resistance and alkali resistance to wool fibers. Intermolecular grafting of wool fiber molecules and intermolecular polymerization with polystyrene was enhanced by high energy radiation from radioactive cobalt and from a 3 million electron volt accelerator in contract research at the Triangle Research Institute in Durham, North Carolina. The grafting could be confined to the surface or promoted throughout the fiber interior by adjustment of the water content.

2. Physical and Mechanical Properties. Advances were made in developing objective physical methods for measurement of fabric and fiber properties to provide evaluations that are rapid and free from human errors. An analog computer was programmed for 21 parameters of wool fiber stress-strain curves. The output of the program roughly agreed with subjective evaluation of wool fabric hand. Advances were also made in measurement of wrinkle recovery using differences in compressibility of sharp triangular creases in fabrics. In addition, a series of wools were assembled varying in yellowness and grayness to establish color standards.

Standard laboratory test methods have proven insufficient to appraise the wrinkling of fabric during wear. Actual wear trials will be necessary to distinguish between fabrics that are close in performance characteristics. Contract research at the Harris Research Laboratories in Washington, D. C. on a method of subjective visual appraisal of photographs of garments which have been worn shows promise of becoming reasonably sensitive. Several

commercial fabrics were obtained and made into men's slacks for a small-scale service test.

Two series of specially constructed fabrics, worsted and woolen, were designed to include variation in construction factors, such as weave, cover factor, weight, etc. These specially designed and constructed fabrics, including wool-mohair combination yarns will be produced and evaluated to determine effects of construction variables on wrinkle resistance.

3. Effects of Radiation and Other Physical Forces on Wool. Basic research is conducted to explain in detail the breakdown of wool by light to provide information that will allow a rational approach to developing methods for controlling light-induced degradation of wool and wool fabrics. Physical evidence was obtained showing that ultraviolet light ruptures disulfide bonds of wool protein producing a cystine free radical and green discoloration. When pure cystine was exposed to ultraviolet light, the same characteristic electron paramagnetic resonance spectrum appeared and a blue color. The green in wool is a combination of the blue from irradiated cystine with yellow caused by exposure of intact wool to ultraviolet light. The ultraviolet-induced green disappears unless the wool is kept under vacuum and protected from oxygen. The green color and the characteristic EPR spectrum for the cystine radical disappear if the wool is allowed to react with oxygen, water vapor, or gently heated in vacuum.

The effects of heat on wool are studied in detail to learn specific causes of wool's instability to heat and to chemical reagents combined with heat. We observed specific chemical transformations or physical rearrangements of the wool molecule. At temperatures near 160° C., protein chains that had not been tied into a crystalline lattice became mobile. New juxtapositions thus became possible for amine and carboxyl groups. New amide crosslinks formed that restabilized the molecular structure.

B. New and Improved Products and Processing Technology

1. WURLAN. WURLAN is the name of the new interfacial polymerization (IFP) application of polyamides to the surface of wool fiber and fabrics. WURLAN-treated wools are in full-scale commercial production and available in substantial quantity in the textile markets today. Research continues to develop improved or less costly interfacial polymerization treatments. Cooperation with commercial operators led to adoption of new chemicals that reduced reagent costs by 50%.

Research is also conducted to apply the WURLAN treatment to wool at the top stage. The successful extension of interfacial polymerization to wool would strengthen wool's competitive position, especially in knit goods and in fabric blends. It would also provide an alternative treatment for tightly constructed worsteds which are difficult to treat as cloth.

Factors of temperature, pH, reagent concentration, and processing speed were evaluated in WURLAN application to wool top. Three treatment combinations showed adequate shrink-proofing combined with low fiber bonding. One was most promising because results were achieved with a short-time immersion treatment. A 600-pound lot of wool top was treated by this procedure for use in collaborative work for the Clothing and Housing Research Division of the Department. For this large research production, a new chemical control of reagent concentration proved effective and reduced the consumption by more than 50%. Such a reduction, if applicable industrially, would significantly reduce the cost of the process.

Important variables affecting processing costs and product quality for wool top must be more fully defined before the WURLAN treatment of top can be considered completely suitable for routine commercial production. Industrial collaboration in studies will expand and further work will focus on choosing among alternative monomer combinations and processing conditions.

Appraisal of alternative reagents for the IFP treatment continues. Improved shrink-proofing of wool fabrics was obtained when strong bases, such as sodium metasilicate, were used in place of carbonate in the diamine solution of the IFP polyamide system. A slight increase in the flat setting of fabric was noted when thiol reducing agents were used. Various polyamides, polyurethanes, and other copolymers are being applied to fabrics and the treated products evaluated.

Blends of coarse wool with kid mohair top were prepared and WURLANized. Blends with 25% and 50% mohair were spun into coarse count knitting yarn and knitted into fairly open structures. Wash tests on the treated knit samples showed little felting compared to untreated samples.

2. Stretch Woolens. An exploratory study of an all-wool stretch yarn is promising. A combination of chemical and mechanical treatments makes wool fibers helical. The helical fibers are elastic even after repeated load cycling and repeated wetting and drying. Experimental knit and woven constructions were strong. The general procedure was to apply twist on twist to a plied yarn, resin treat, and then back twist past the zero point to a standard twist. IFP and application of preformed reactive polymers both were used to set high twist yarns. Polyamides, polyurethanes, and copolymers applied by IFP and reactive polyethylene resin showed promise. The permanence of the treatment is being tested. The laboratory will produce enough yarn to make fabric for evaluation.

3. Yarn and Fabric Construction. Both yarn and fabric construction greatly affect the performance of fabric and its response to chemical treatments which make care easier. The effect of variations such as weave, cover factor, weight, etc., of worsteds and woolens wrinkling during wear is being investigated in contract research at the Harris Research Laboratories in Washington, D.C. Emphasis to date has been on the development of evaluation techniques (see paragraph A-2).

The mechanism of lubrication of worsted yarns is being investigated by the Hosiery and Allied Trades Research Association in Nottingham, England under a P.L. 480 grant. Work continues on the influence of different amounts of added wax upon frictional properties of the yarn. Apparatus for waxing yarn, knitting it, and measuring frictions involved was modified to make a kinetic yarn-to-yarn friction measurement. Experimental methods and equipment are still being developed in this study and no conclusions can be drawn yet.

We have been trying to discover if WURLAN treated top is suited to the mechanics of ordinary processing. The adhesion of one fiber to another in treated top ranged from satisfactory to highly unsatisfactory depending upon the treating conditions (reagent concentration, time, temperature, and pH). Resin dusting during the processing increased as reagent concentration, temperature, and pH increased. Dusting decreased with longer immersion time. Small lots (10 lbs.) indicated too high pH, temperature, or reagent concentration made the top extremely difficult to draw and spin. The recombing noilage of treated top was not excessive. A dye absorption procedure was found to measure the resin loss during processing. Indications are that this analysis will be a useful tool for controlling production operations.

4. Fiber and Fabric Treatments to Make Care Easier. The commercial success of interfacial polymerization (WURLAN) does not obviate other finishing treatment for wool. Several promising leads for continuous single application, multiple purpose finishing agents are being investigated. Some of the most promising are fluorine-containing synthetic compounds which impart shrink resistance as well as resistance to water and oil. A number of copolymers and long-chain fluorochemicals were synthesized and subjected to preliminary evaluation on wool fabrics. The screening process will continue and the most promising reagents will be subjected to more extensive laboratory evaluations.

Reactive polyethylene finishing resins of two types were screened: reagents used in organic solvents by a dip-pad cure process and those applied in water emulsions by a new crosslinking technique. Both types protected wool against shrinkage at uptakes of reagents as low as a half percent. Application from water emulsion appears adaptable to single-step preformed polymers continuous processes. Water emulsion application of other synthetic chemical reagents is being studied. Products based on new, relatively inexpensive polyethylene resins may extend the range of polymeric finishes.

Treatment of wool with ozone as briefly as 1-1/2 minutes stabilizes the fabric against felting shrinkage. The ozone-treated wool dyes and bleaches faster than untreated wool and retains tensile strength. Fabric abrasion resistance is somewhat lower. The cost of a continuous ozone shrink-resistant treatment appears very low and pilot equipment is now being designed to take this laboratory observation one step further toward commercial use.

A process for high-energy radiation-induced grafting and polymerization of styrene on wool is being investigated by the Research Triangle Institute at Durham, North Carolina. They have discovered basic information on the effects of dose and dose rate on the grafting of polystyrene to wool in the presence of high-energy radiation (see paragraph A-1). They showed that cloth made from grafted fiber lost superficial water faster than untreated cloth and that water diffused into and out of the treated fibers considerably faster. Such a treatment shows definite promise as a practical method for improving drying rates for wool fabrics. Research to date indicates that the radiation grafting may be induced at sites in wool associated with discoloration. If further research bears out these observations, it is possible that such modified wools would have a greater chemical stability to radiation including visible light. Properties of wool fabrics that have been treated by the radiation-induced grafting of styrene, vary considerably with the degree of swelling at the time of treatment. The swelling can be influenced by control of the moisture content of wool. It appears feasible to produce fabrics with a wide range of drying rates to meet specific requirements.

5. Wool Discoloration and its Control. The yellowing of wet baled grease wool is a problem of considerable economic interest. One hundred pound bales of grease wool treated with 1% paraformaldehyde showed little change in temperature or color after three weeks' aging, although the initial moisture content of the baled wool was as high as 40%. Similar untreated bales increased in temperature from 74° F. to 100° F. in five days with a 40% increase in yellowing. Yellowing depended upon moisture content of the wool and there was no discoloration in untreated samples below about 15% moisture. Microbial activity was not found to be the direct cause of yellowing of moist grease wool. However, microbial activity heated the wool and increased rate of yellowing at the higher moisture levels. The paraformaldehyde prevented microbial action.

Contract research was completed on improved bleaching processes for wool at the Lowell Technological Institute Research Foundation in Massachusetts. The research indicated ways to increase brightness of wool through bleaching with peroxide and conditions for minimizing alkali solubility and strength loss. The unscourable discoloration in wool can be bleached, but only at the expense of some damage to the fiber. Chemical crosslinking treatments can reduce the damage, but these treatments in turn reduce wool's abrasion resistance. Crosslinking reagents were evaluated for this purpose. Formaldehyde effectively inhibited the increase in alkali solubility which is otherwise caused in the wool by peroxide bleaching. Additional peroxide was required to compensate for losses through reaction with the formaldehyde. The contractor's recommendation was that wool should be bleached with hydrogen peroxide at pH of 9 to 10 in the presence of tetrasodium pyrophosphate for about 5 to 10 minutes at temperatures between 160 and 180° F. in a continuous process. It was further recommended that alkali solubility of wool

be minimized by pretreatment with alkaline formaldehyde or by addition of formaldehyde directly to the bleach bath.

6. Improved Finishing Treatments for Wool Fabrics. The consequences of interrelationships of different wool finishing processes are being investigated by the Textile Research Association in Helsinki, Finland under a P.L. 480 research grant. This research deals partly with top dyed fabrics and partly with fabrics to be piece dyed. Both kinds of cloth include plain, Panama, and twill weaves made from coarse, medium, and fine wool. Preliminary and pilot plant experiments carried out on fabrics to be piece dyed included scouring and setting treatments prior to dyeing as well as dyeing experiments. The development of proper finish in wool fabric begins early in the processing. Scouring should produce the highest possible degree of homogeneity of fibers in order to improve fabric dyeability. The chemical setting of fabrics must be thorough prior to dyeing and dyeing should be carried out at a low pH. Experiments with simultaneous dyeing and setting resulted in smooth fabric surface but poor dye penetration. Cooperation with the Norwegian Textile Research Institute makes possible simultaneous setting and dyeing in commercial beam dyeing equipment. Much is still to be done to establish the best conditions for both setting and dyeing.

Experiments with top dyeing are also being conducted. The various fabrics of this experiment were each dyed and finished by conventional methods for purposes of comparison with experimentally finished fabrics. With conventional methods, crease recovery values of piece dyed fabrics were always more flexible, more easily elongated by low load than top dyed fabrics. The differences between piece-dyed fabrics and top-dyed fabrics were less in coarse fabrics than fine cloth.

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AREA NO. 5. CITRUS AND SUBTROPICAL FRUITS--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The economic stability of the citrus and subtropical fruit industries in the Western Region is dependent upon effective utilization of fruit that cannot be accommodated on the fresh fruit market. The utilization of surplus or wholesome but blemished fruit provides the margin necessary to assure adequate returns to the farmer and continued development of stable markets. Ineffective utilization of products and continuously increasing processing costs are resulting in decreased returns to the growers. The California-Arizona grapefruit industry is encountering difficulty in disposing of both fresh fruit and processed grapefruit products. The pineapple and subtropical fruit industry in Hawaii must find practical methods for processing its products for export in order to prevent the accumulation of burdensome surpluses. The navel orange industry in California is hampered by the unavailability of satisfactory processes for the utilization of navel oranges. Juice extracted from early fruit, and during some seasons from almost all of the navel oranges, contains unknown substances that impart an intolerable bitter flavor to juice products after mild heat-processing or after standing at ambient temperature for a short time. Large new plantings of navel oranges may be expected to aggravate the utilization problem. Deterioration of the flavor and color of these and other processed citrus and subtropical fruit products imposes severe limitations upon the economic stability of the industry.

Information is needed on the chemical composition of citrus and subtropical fruits and their products and byproducts as a basis for the development or application of new and improved methods of processing; and for the production of new and improved food and industrial products and pharmaceuticals. Special attention needs to be given to the nature of the chemical changes involved during pre-treatment, processing and handling which lead to the formation of off-flavors, -colors, and -odors in processed products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a concentrated program of fundamental research on citrus and subtropical fruit and its application to industry problems is conducted at the Division headquarters at Albany, California; at the Fruit and Vegetable Chemistry Laboratory in Pasadena, California; at the University of Hawaii, Honolulu; by contract at Tucson, Arizona; and, under a P.L. 480 grant, in Bogota, Colombia. Investigations are conducted on the composition of citrus essential oils, flavonoid compounds and other citrus constituents that are related to off-flavors and darkening of citrus products, the natural flavor components of oranges, enzyme systems that are involved in the appearance and disappearance of constituents and structures of plant tissues, constituents of dates that affect the quality and stability of products, and the application of findings of such research to the development of new and improved citrus, tropical, and subtropical fruit products.

The Federal program of research in this area totals 20.7 professional man-years, including contract research equivalent to about 0.4 professional man-year per year and one scientist whose salary is provided under Memorandum of Understanding by the Lemon Products Technical Committee. Of the total, 14.3 are assigned to investigations on chemical composition and physical properties and 6.4 on new and improved food products and processing technology. In addition, the Division supervises a research project on the development of new tropical fruit products supported by a P.L. 480 grant.

PROGRAM OF STATE EXPERIMENT STATIONS

The states are engaged in research on the utilization of citrus and subtropical fruits in an effort to expand markets through increased use. Research on citrus begins with efforts to reduce decay during storage and transit through control of the physical, biochemical and physiological changes in citrus during handling--i.e., the effects of precooling and study of factors of temperature, humidity and air flow during cooling.

New product studies involve determination of the basic product characteristics, flavors, types and components and relate these to market demands. A thorough examination of the sources of flavor of some common foods including citrus is in progress. A phase of this program deals with the effects of oxidation and/or hydration on the flavor and aroma of the terpenes of citrus and the role structure plays in odor production. Conversion of citrus terpenes to useful chemical compounds is also under study. Another study has as its goal to extract, separate, identify and determine quantitatively each of the volatile components responsible for the natural flavors and occasional off-flavors in citrus fruits, citrus oils and processed citrus products.

The characteristics of commercial frozen Florida orange concentrate and superconcentrate are frequently determined to establish physical and chemical characteristics of the products. Base juices are prepared from citrus fruits and used to determine the effects of the fruit components on the characteristics of frozen citrus concentrate.

Firming of canned grapefruit sections with calcium salts and other materials is also under study. Characteristics of canned and concentrated juices are determined initially and after storage at elevated temperatures.

Utilization of citrus waste is receiving attention. In one study, isolated cultures from natural sources are being used to investigate production of glycerol and glycols from citrus wastes by fermentation.

The program with other subtropical crops such as guava, mango, soursap, banana, pineapple, coffee, and plantain includes production of freeze-dried products of high quality and good storage life. The economic feasibility of expanding markets for subtropical agricultural products through processing and utilization of new products is being further tested by preparation

of soft drinks from tropical fruits--carbonated and non-carbonated, canned and bottled. Basic biochemical and microbiological studies of tropical foods are directed to discovery of special methods, special properties or nutritional qualities which may be used in new product development. Production of such products as banana purée, fried snack items, flakes, flours, fruit powders, flavoring extracts, candied items, canning syrups, nectars and juices is under study.

In addition, attempts to isolate the enzymes of fig latex are underway. The isolated proteolytic enzymes are characterized as to molecular weight, activity and amino acid composition.

Two stations, Hawaii and Puerto Rico have programs designed to improve the economic condition of their coffee industries. These researches embrace work on the microbiology of the coffee fermentation process, on the drying of coffee, and on the quality and acceptability of the final product.

The total research effort in citrus and subtropical fruit is about 21.6 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Citrus Composition. The quality and stability of citrus products are determined by the chemical components and physical properties of the fruits from which they are made. Research is continuing on composition of grapefruit, cold pressed lemon oil, and lemon juice concentrate. Research on lemons is supported in part by the Lemon Products Technical Committee which pays the salaries of two research fellows at the Pasadena Laboratory. Research on grapefruit oil disclosed the presence of a sesquiterpene ketone (nootkatone) at significant levels. This ketone appears to be an important factor in grapefruit flavor. Threshold levels of nootkatone for taste detection in a sucrose solution were determined organoleptically to be around 100th of the normal concentration of the compound in high flavor quality grapefruit oil. Nootkatone was found in the peel oil-free juice at about the threshold level. The compound was also found in other citrus oils but in lower concentration.

Improved chromatographic procedures confirmed the presence of two sesquiterpenes earlier reported in lemon oil (beta-bisabolene and alpha-bergamotene) and also indicated the presence of caryophylene and five sesquiterpenes in minor amounts. Studies of the amounts and identities of the carotenoid pigments of lemon peel and pulp at various stages of maturity and lemon leaves and frozen juice concentrate were completed. These substances contribute to the color and nutritive qualities of lemon products. Each of these sources contained at least 20 individual pigments although there were major differences in the pattern of pigments from each source. Lemon peel contained higher percentages of violaxanthin and zeta-carotene and smaller

percentage of cryptoxanthin than did the pulp. The pulp also contained several carotenoids not present in the peel. Beta-carotene was the major carotenoid of frozen juice concentrate. The yellow color of ripe lemon peel is due to the small amount of carotenoids and the light color of the major carotenoids. The major carotenoids of green lemon peel and leaves are those usually associated with photosynthetic tissues.

A study on the effects of climate and growing area on the composition of lemons is continuing in order to determine the range of natural variation in composition over several growing seasons. The completed data will be useful in improving the accuracy of previously developed analytical methods coupled with multiple regression analysis for determining the authenticity of lemon juice and useful to growers and processors in defining factors influencing fruit composition. Individual polyphenolic compounds of lemon juice are being isolated, identified, and their concentration determined.

Carotenoid pigments of desert grapefruit, Marsh seedless and Ruby Red, are under intensive study. Late season fading of red from Ruby Red grapefruit pulp was found connected with the formation of beta-carotene and its diepoxide at the expense of lycopene. Phytoene is one of the major carotenoids in the Ruby Red grapefruit but is not present in detectible amounts in the Marsh variety. As grapefruit ripens, green chlorophyll disappears and the carotenoid pattern of both peel and pulp changes significantly. The carotenoid mixture in peel is extremely complex. A total of 23 individual components were found in the peel of green Marsh seedless grapefruit and a total of 34 components in the peel of ripe fruit. During ripening major changes in relative concentration of important carotenoid constituents took place. Studies were initiated on steroids and coumarins of desert grapefruit. Isolation and accumulation of individual compounds of these types will be made for identification and tests of biological activity.

2. Bitter Constituents of Citrus. Several classes of compounds that are natural constituents of oranges and grapefruit impart bitter flavor. During processing and in subsequent handling and storage, changes may occur in individual components that affect quality of product. A clear understanding of the chemistry of these compounds is a first step toward improvement in products and processes. A number of carbon-linked flavonoid glycosides were isolated from citrus fruits and the structure of one of them (vitexin) has been elucidated. It will help clarify the chemistry of the entire group. An important disaccharide (neo-hesperidose) was synthesized. This compound is responsible for extreme bitterness or sweetness when it exists linked to certain flavonoids. The synthesis of neo-hesperidose has proved correct a structure previously hypothesized from degradative methods. Furthermore, it will make possible preparation of this disaccharide or its glycosides in radioactive form for metabolic studies.

The bitter flavonoids neohesperidin and naringin were previously converted to dihydrochalcones that are sweet enough to be considered as commercial

sweeteners. Neohesperidin has now been synthesized from readily available commercial chemicals. The synthetic pathway used has been adapted to other syntheses and new flavonoids are being constructed in the laboratory and evaluated for bitterness and sweetness in a search for still better compounds that might be used as low calorie sweetners for food.

Limonoid compounds responsible for the bitterness of navel oranges are being isolated and identified. A new bitter principle was isolated in pure form from several citrus fruits and its structure established by infrared and nuclear magnetic resonance spectra and by chemical conversions. The new compound (deacetylnomilin) is closely related to obacunone and nomilin which are intermediates in the formation of the established bitter principle, limonin. The new bitter principle can be converted by acetylation to obacunone and nomilin. Thus these three compounds constitute a pool of interconvertible metabolites which exhibits different bitterness depending upon the relative amounts of each as they exist in navel orange juice at any particular time. Larger quantities of the new bitter principle are being isolated for more detailed study.

3. Pharmacological Investigations of Citrus Products. The dihydrochalcones of bitter neohesperidin and naringin from citrus are intensely sweet and are potentially useful as low calorie sweeteners of food products and for use in sugar-free diets. Ninety-day feeding tests of rats were concluded, indicating no obvious toxicity at levels of ingestion up to 200 times the level calculated to be equivalent to the sweetening provided by the average daily sucrose consumption in man in the United States. The rats used in the 90-day feeding tests were also used for a reproduction assay. The safety of these compounds for use by man will depend upon the absence of unfavorable histopathological findings.

4. Fruit Flavor Components. Gas-liquid chromatography continues the rapid advance of the past several years and continues to expand its usefulness in study of the chemistry of volatile natural components. Information was obtained on relative efficiency of capillary columns attached to hydrogen flame ionization detectors and attached directly to the mass spectrometer. Operation with the end of the column at the mass spectrometer vacuum did not reduce efficiency. Large bore capillary columns and packed columns with high efficiency and a low pressure-drop were developed to prepare pure samples of compounds heretofore very difficult to isolate. Spectral data in addition to mass spectra are useful to establish chemical structure of difficult compounds and mixtures. Milligram quantities of pure material must be isolated to observe and measure such spectra and these new highly efficient columns will help prepare such samples. Larger samples are also required for essential taste testing of the odor contribution of the individual compounds.

Evidence is accumulating that the more important characteristic aroma-bearing compounds of oranges are oxygenated compounds which boil well above 100° C. Earlier chromatographic systems did not separate high boiling mixtures well. The new packed columns with low pressure-drop are already proving to be extremely useful particularly in recovering material for nuclear

magnetic resonance analyses. Computer analysis of nuclear magnetic resonance data can draw a conclusive spectrum from much smaller samples. It was this reduction in sample requirement combined with the large bore capillary and low pressure-drop chromatographic columns that allowed substantial advances in the separation of high boiling oxygenated compounds from orange oil.

5. Composition of Dates. Utilization research on dates is conducted with industry support by means of a fellowship provided by the Date Administrative Committee coperating under a Federal Marketing Order. In compositional studies related to discoloration of dates and date products, dactylifric acid was found to be one of the main enzymatic browning substrates. Isolation, structural determination, and synthesis proved for the first time the existence in nature of a new family of compounds, the substituted cinnamoyl shikimic acids. Knowledge of these new compounds opens the way for more detailed studies of the browning mechanism of dates. The identification of dactylifric acids may be important to the biochemistry of plants in general because of the importance of shikimic acid in the biosynthesis of aromatic compounds such as tyrosine, phenylalanine, substituted cinnamic acids, flavonoids, coumarins, and lignin. Isodactylifric acid, an isomer of dactylifric acid, was isolated from dates in pure crystalline form and its structure determined. This compound also has proven to be an important enzymic browning substrate.

B. New and Improved Food Products and Processing Technology

1. Citrus Products. Large quantities of the sweet compounds naringin dihydrochalcone, and neohesperidin dihydrochalcone were prepared from citrus byproducts and found to have no obvious toxicity to rats in 90-day feeding tests at levels of ingestion up to 200 times the sweetening provided by the average sucrose consumption of a man in the United States. If research evidence confirms the safety of these intensely sweet compounds they may find use in dietetic foods and soft drinks and as sweeteners for diabetics. The compounds are up to 20 times as sweet as saccharin and are free from the bitterness of saccharin. Considerable commercial interest has been expressed in the sweeteners. Tests by one company indicate that sweetness from these compounds is relatively slow to develop in the front part of the mouth as when used in coffee, for example. The company believed they would serve better in chewing gum where the sweetness may take longer to develop in the back part of the throat. The conversion of naringin into new flavanones and dihydrochalcones will continue with the emphasis on developing new sweeteners that sweeten faster and in the front part of the mouth.

Studies were continued on the effect of growing area, culture, and processing on lemon juice composition in order to be able to identify the natural product. Commercially feasible variations in processing did not alter composition appreciably. Citric acid content of various juices calculated using the multiple regression equation reported last year agreed quite well with the titrated acidity for each processing variable. Knowledge on

natural variations in the composition of lemons is accumulating and will improve the reliability of recently developed methods for characterizing juice products, and will define for growers and processors the factors which influence the composition of lemons.

2. Date Products. Research and development on improved date products are supported in part by the Date Administrative Committee operating under a Federal Marketing Order. Texture improvements by a controlled heat process were reported last year and have become widely used in date packing. Heat and moisture activation of natural date enzymes results in sucrose inversion, increased tenderness, and improved quality, and inhibits the tendency of dates to dry out during storage.

Other factors important in the quality of date products are under continuing investigation. A tendency of dates to turn dark during storage can be inhibited by protecting the dates from oxygen. Studies on the flavor of processed dates and flavor changes which occur during processing were initiated. In commercial experience some lots of processed dates taste better than others, but no one knows why. We suspect that the more acid dates have more flavor and are making progress on modified processes that increase acidity of dates. Preliminary observations indicate that flavor enhancement is associated with, but not necessarily dependent, upon increased acidity. Studies on flavor development and enhancement in processed dates will continue.

3. Tropical Fruit Produc . The field station established in Honolulu last year in cooperation with the Hawaii Agricultural Experiment Station has initiated research on bulk- and weight-reduction of tropical fruits to yield high-quality products that ship well for export to the mainland or foreign countries. A Department scientist was transferred to the new station and new laboratory facilities were provided. A preliminary exploration of processing properties of a variety of Hawaiian-grown tropical fruits was conducted. Juices and purées were prepared from guava, papaya, passion fruit, pineapple, and jaboticaba. Heat treatments are being sought to inhibit the strong gelling of guava and papaya. Flavor investigations were initiated to determine the differences between freshly pressed pineapple juice and the canned pineapple juice that is widely known and accepted. Characterization of the carotenoid pigments that color guavas pink is under investigation. Effects of drying rates on product color of dehydrated products from different varieties of bananas were also initiated. Improved means for retention or restoration of the flavor and color qualities of tropical fruit products will continue.

Research on stabilization of flavor concentrates of tropical fruits was initiated at the Institute of Technological Investigations in Bogota, Colombia, under P.L. 480 funds. Equipment was obtained for pilot extraction and concentration of juice and for laboratory analysis.

4. Foam-mat Drying. Cooperative research with the Southern Utilization Research Division is conducted on the foam-mat drying of orange and other citrus products at Winter Haven, Florida. Informal cooperation continues with industry representatives who are interested in commercial application of foam-mat drying. Commercial production of foam-mat dried lemon juice without added sugar is underway in California.

Improvement in foaming procedure allowed a longer beating time under conditions that do not destroy the foam and resulted in the drying of orange concentrate to 1% moisture content or less. Such low moisture content allows packaging and long-term room temperature storage without the use of in-package desiccants for dried orange products. The powder can be packed more tightly by pressing it through warm rollers. Improved release agents were found that prevent the powder from sticking on the warm rolls. Incorporation of homogenized and heat stabilized orange peel with orange juice concentrate and a small amount of sugar (5% of solids) yielded a high flavor quality foam-mat dried orange juice without the "locked-in" peel oil for flavor fortification.

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AREA NO. 6. DECIDUOUS FRUIT AND TREE NUTS--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. Fruits and nuts are valued for their unique flavor, color, and natural vitamin content. In the period of abundance at harvest time, markets are glutted and growers often do not get an adequate return. Crops are perishable, and processing to preserve their unique qualities is difficult. No processed fruit retains completely the fresh values, although many highly acceptable products exist and about half of the fruits and nuts marketed in the United States are processed. Processing makes these commodities available to consumers the year around, and has opened new markets for producers. The proportion of processed commodities is steadily increasing but is dependent upon a continuing flow of new knowledge. Processing to preserve color, flavor, and texture presents many problems generally, and each new product requires the application of much scientific and technological skill.

The freezing process for preserving certain fruits keeps the products excellent at near fresh fruit condition. In spite of the gains in quality realized in freezing, many unsolved problems remain. The enzymatic browning of frozen peaches and the sloppy texture of frozen strawberries on thawing are two good examples.

Frozen fruits require expensive low-temperature storage and transportation facilities. The expense is greatly reduced by removing a portion of the water from the products. Orange and other fruit juice concentrates are well established in U.S. markets, and dehydrofrozen apple slices (rapid drying to 50% bulk weight and then freezing) are just becoming well established. Many other fruits and fruit juices should be amenable to concentration. Products of this type, however, are not so well adapted for export as those which do not require refrigeration.

The maximum weight reduction can be achieved through dehydration. The drying of fruit juices has been successfully accomplished by the vacuum puff drying and foam-mat drying processes. The latter is under intensive study, because it can be carried out at atmospheric pressure and consequently offers economy in processing. This process must be worked out in detail for many, as yet untried, fruit purees and juices and on pilot-plant scale for those products that show promise. Flavor recovery and the incorporation of recovered flavor in solid carriers for addition to the dried products require technological and basic chemical study. Essence recovery techniques developed for fruit juice concentrates are not completely satisfactory for this purpose.

Dried and canned fruits are now widely used in the U.S. and abroad. The popularity of dried fruits overseas and in this country would grow if stable, higher moisture dried fruits were available and if lower levels of sulfur

dioxide could be used without loss of quality.

Container costs for canned fruits limit the shipment of these products overseas. A solution of the container problem may be found in the use of lightweight fiber, foil, or plastic containers and aseptic filling procedures.

Fruit growers need new varieties of tree fruits and berries suited to processing and resistant to diseases endemic to each region of production. Utilization research is required in cooperation with farm research to assure growers of a market for fruit in the processing industry.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research on deciduous fruits and tree nuts is conducted at the Division headquarters at Albany, California; in field stations at Pasadena, California, Prosser and Puyallup, Washington; by contract in Honolulu, Hawaii; by grant at Cambridge, Massachusetts; and by grant funds under P.L. 480 in Israel and India. Fundamental research is conducted on fruit constituents that are involved in the flavor, color, and texture of fruit products, and includes development of laboratory tools to isolate and characterize individual components, investigation of such components as they occur naturally and as they are altered by operations involved in preservation, and the relationships between the components and the product values being preserved. Applied research is conducted to develop new and improved processes and products that will increase utilization of fruits and tree nuts, including the development of high quality concentrated and dehydrated products and more stable shelled tree nuts and the selection of improved processing varieties. Pioneering research on plant enzymes is also conducted.

The Federal program of research in this area totals 48.2 professional man-years, including two scientists whose salaries are provided by two cooperators (Dried Fruit Research Advisory Committee, whose membership represents the California Raisin Advisory Board, the Dried Fig Advisory Board, the California Prune Advisory Board, and the Dried Fruit Association of California; and Diamond Walnut Growers, Inc. - one each), under Memoranda of Understanding; one grant providing research at a rate of approximately 0.5 professional man-year per year and four contracts providing research at a rate of approximately 2.6 professional man-years per year. Of this number, 26.7 are assigned to investigations on chemical composition and physical properties; and 21.5 on new and improved food products and processing technology. In addition, the Division sponsors basic research on fruit by means of two P.L. 480 grants.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations are engaged in a comprehensive program involving both basic and applied research on fruit processing and products. Evaluation of fruit varieties and selections is a necessary service for the breeding programs. Additionally the relationships of variety, other production and cultural practices, method of preparation, and processing procedures to quality and utilization of the finished product are determined. Application of mechanical harvesting is spreading rapidly, and makes it necessary to evaluate how mechanical harvesting affects the utilization of the harvested fruit. Research is also in progress on the identification and characterization of changes associated with post-harvest storage and ripening. The aim is to elucidate the metabolic reactions associated with ripening with a view to their ultimate control. Degradative and structural changes are receiving most careful attention. In some cases the respiratory activity of the fruits is being measured to guide development of holding and packaging requirements.

Work on the chemical composition and physical properties of fruits involves studies of a number of fruits. For example, the non-volatile organic acids and sugars of grapes are being determined. Other studies involve the biochemistry of the color and pigments of fruits. Basic research is in progress on identification of the polyphenols of fruits and the role they play in enzymatic browning. Attempts are being made to isolate and identify the naturally occurring phenolic substances of commercially important fruits which affect the acceptability and stability of fruit juice products, wines, and dried and frozen fruits. Advances in methodology and biochemistry of plant tannins, leucoanthocyanins and related flavonols are being made. The chemical and physical changes of pectic substances before and after processing and during storage are related to texture and reconstitution properties of fruit products. It has been found that ethylene is important in the formation of peroxides and further investigation is concerned with the role of the lipid fraction in ripening of fruits.

Characterization of fruit flavors is being pursued by improved techniques of gas chromatography. Compounds are being identified and effort is being devoted to determining their significance in flavor of the fruit. Flavor variations and off-flavors are being studied also.

Investigation of enzymes specifically involved in the formation of off-flavors in frozen fruits is in progress. Studies of enzyme mechanisms and properties constitute an important fundamental phase of the fruit investigations. Browning problems and control of enzyme reactions are other facets of enzyme systems under study. Some effort is being devoted to synthesis of flavor compounds and pigments through use of extracted natural enzymes. Other analytical work involves determining flavor, texture and nutritional qualities.

The objectives of the microbiology program in fruit utilization vary from determination of the occurrence of certain organisms to study of yeast

growth factors important in the wine fermentation. Microbiological spoilage receives careful attention. The use of vitamin K_5 and its effect on the various food spoilage organisms is under study. Basic investigations of the ecology, taxonomy and physiology of yeasts and molds involved in food spoilage are made to better understand how spoilage microbes occur in nature and how they may be controlled in food products. A highly specialized study relates to the microbiology of olive fermentation and spoilage. Other research is concerned with the evaluation and enumeration of bacteria found in frozen fruit products and developing methodology for identifying certain groups.

Research directed to development of new processing technology is a major component of the fruit utilization program. Study of the influence of maturity, post-harvest handling, storage and ripening procedures and processing methods on the quality of canned pear products is an example of a fully integrated project. Comprehensive studies dealing with the thermal processing of a number of fruits are in progress. Basic studies relate to mechanism of heat transfer, the effects of thermal processing and the mechanism of thermal breakdown of various constituents, i.e., fats, proteins, carbohydrates and heat labile vitamins. Methods, equipment and layout of processing lines also receive attention. Methods of freezing, dehydrofreezing, freeze-drying, irradiation and dehydration of fruits are investigated. The effects of the process on organoleptic, physical and chemical characteristics of the fruit are measured. For example, the optimal conditions for dehydrofreezing red cherries are being determined and the dehydrofrozen cherries are being evaluated for use in pies. Investigation of the effect of ultrasonic energy on freeze-drying rate is studied through consideration of the kinetics and mechanisms of energy and mass transfer. Effects of chemicals, hydrocooling, refrigerated storage and controlled atmosphere storage and holding are also under investigation. Because many of the changes in foods relating to processing methods are textural, a fundamental study of the influence of processing on microscopic structure of foods is in progress.

The objective of the product work is to develop new or improved food products. Development of processes or products to improve the utilization of fruits involves work on dehydrated fruits, i.e. prunes; apple juice; apple sauce; frozen fruit pies; apple-fruit juice blends; sherry wines; brined cherries; canned apple slices; low sugar apple jelly; macadamia nuts; peaches and grape products. Factors affecting fluidity, plasticity, consistency, shape, flavor, appearance, texture, physical, chemical and organoleptic properties are being studied. Total professional man-years equals 57.3.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fruit Pigments. Red, purple, and blue anthocyanin pigments are responsible for color of many fruits. The changes that occur in these pigments during processing and storage adversely change the appearance of their

products. Basic studies of the nature of anthocyanin pigments are continuing. Studies of model chemical systems of flavylium salts are revealing the course of chemical modification of this type compound and pointing the way to procedures for stabilizing fruit color. A significant finding of these studies previously reported was the oxidative rearrangement of flavylium salts to 2-aryl-substituted benzofuran derivatives. One of these new compounds, coumestrol, has estrogenic activity and has been isolated from alfalfa and other legume forages. The conversion of flavylium salts to coumestrol compounds was also demonstrated in laboratory animals.

Model anthocyanidin pigments were decolorized in laboratory studies by reaction involving other phenolic compounds. For example, such phenolic compounds as fluoroglucinol, resorcinol, and catechin which occur in most plant extracts rapidly reduce flavylium salt to a colorless flavin. Although it is commonly assumed that oxidation of anthocyanins is responsible for most color loss in fruit products, this newly observed phenol reaction strongly suggests that decoloration of anthocyanins might be due at least in some instances to a reduction of the pigment by ubiquitous phenolic compounds. Whereas these studies involve only model chemical systems, research will ultimately seek to find if this reaction occurs in fruit juices. If it does, a number of new approaches to color stabilization should be considered.

Basic research on leucoanthocyanins and related phenolic pigments in fruits was initiated by contract at the University of California at Los Angeles. The studies will include the biological formation and changes of leucoanthocyanins and related flavonoid compounds in fruits to determine the nature of the oxidative and other reactions responsible for changes of these chemical compounds during processing and storage. A related project on the chemical behavior of leucoanthocyanins in fruits was initiated at the University of Delhi in India under Public Law 480 funds. Mechanisms, such as reactions with sulfur dioxide, involved in color changes will be studied to develop means for controlling darkening and other color deterioration in processed fruit. Pigments were extracted from the peels of apples, pomegranates, and peaches and found to contain appreciable amounts of leucoanthocyanins. Isolated leucoanthocyanins from these sources are being further characterized and studied.

2. Enzymatic Browning of Fruit. Basic research on a methyl transferring enzyme led to a procedure for preventing discoloration of cut apple surfaces. Observations on the apple tissue suggested an enzymic action similar to methyl transferase systems previously isolated from animal liver tissue. Further study revealed plant sources of such an enzyme. O-methyl transferase was easily extracted from pampas grass which is a possible source of the enzyme as a chemical reagent. A number of important phenolic metabolites of plants and animals were tested and methylated through the action of the enzyme. A quantitative radiochemical assay of high sensitivity was worked out to test protein fractions for methyl-transferring activity. The new analytical procedure was used in isolating purified enzyme fractions from protein extracts. Studies of the specificity of purified enzyme fractions

can now be conducted. In addition to the potential of preserving color of cut fruits, this basic research has important implications on the organic synthesis of methoxy phenolic compounds. Several fruits were tested for the presence of O-methyl transferase activity and found negative. The absence of this enzyme implies the necessity of developing a suitable reagent enzyme from other sources.

Related studies on enzymic browning of fruits are conducted at the Hebrew University in Jerusalem, Israel, under P.L. 480 grant funds. Electrophoretic separation of enzymes from apples was advanced. Several components with phenolase activity were isolated and studies were conducted on the inhibition of phenolase by chemical reagents. An inhibitor, highly specific for phenolase, showed that peaches contain a soluble enzyme that oxidizes phenols but is not inhibited as are other phenolases. With the next harvest of peaches this interesting enzyme will be studied in greater detail. In addition, peaches were found to contain other phenolases that resemble in all essentials the enzymes that were found previously in apples. A soluble apple enzyme that was not found in unripe apples apparently is formed or liberated from particulate fractions during ripening. Studies on this enzyme will continue in order to follow its development in ripening apples including studies in which ripening will be accelerated with ethylene. The properties of this enzyme will be investigated if it is found present in sufficient amount for isolation.

3. Chemical Origin of Plant Structural Tissue. The biological pathways whereby polysaccharides of cell wall and other structural tissues are formed and degraded in fruit are under investigation because such compounds affect the texture of fruit and fruit products. A searching investigation of biochemical interrelationships between cell wall polysaccharides and myo-inositol (a simple carbohydrate compound found widely in nature) has revealed an important chemical process that involves the positioning of elements within these molecules. Radiotracer techniques were used to follow the production of D-xylose by ripening strawberries. These studies explained the conversion sequence from myo-inositol to D-glucuronate to D-xylose.

The study on the conversion of myo-inositol to cell wall polysaccharides and related carbohydrates in fruit tissue revealed this compound as a potent tool for basic study of cell wall components, especially pectin and hemicellulose. When myo-inositol is specifically tagged with radioactive elements the label appears specifically in uronic acid and pentose residues of the cell wall polysaccharides. It is thus possible to insert radioactive labels in certain components of the cell wall to the exclusion of other carbohydrate components such as cellulose. The selective radioactive tagging should be useful for studies of processing and storage variables on the texture influencing components of fruit tissue.

A related research grant was initiated at Harvard University to seek information on the formation and physical structure of fruit cell walls. They are now undertaking detailed histochemical investigations of plant cell wall

structures. A new tissue fixative, glutaraldehyde, was used in sample preparation and initial studies gave evidence for the first time of an ordered array of intracellular microtubules just inside the cell membrane in a wide variety of plant tissues. The observation provides the first glimpse of an ordered pattern of cytoplasmic processes conceivably responsible for ordered cellulose fiber deposition in the cell wall. Further, it offers a rational phylogenetic explanation for processes related to protoplasmic streaming, since the microtubules of these plant tissues seem to resemble the cilial processes of bacteria and animal cells. The functions of the microtubules in plant tissues will be explored in depth as this project continues.

4. Pharmacological Investigations--Deciduous Fruit. Treatment of high-moisture dried fruits with ethylene oxide has been reported to result in residues of ethylene and diethylene glycol. Approval by Food and Drug Administration for use of ethylene oxide requires the development of an analytical method for the glycol residues, so they may be measured and controlled at non-toxic levels. It was demonstrated that when known amounts of ethylene glycol, the principal breakdown product of ethylene oxide and water, were added to high-moisture dried fruit that had not been treated with ethylene oxide, only 75-85% of the glycol could be recovered. A paper-chromatographic isolation technique has now been developed which makes it possible to quantitate the ethylene-oxide residue in high-moisture dried dates.

The enzyme O-methyl transferase has been found effective in preventing enzymic discoloration of cut and peeled fruit. Enzyme-substrate studies are under way in biological systems. A variety of orthodiphenolic compounds, including phenolic acids, phenols, coumarin, and flavonoids were found to be suitable substrates for this enzyme.

5. Fruit Flavor Components. Refinements in gas-liquid chromatography (GLC) continue to advance chemistry of volatile components of fruits. Large-bore capillary columns and packed columns of low pressure-drop and high efficiency were developed to prepare pure samples of compounds heretofore very difficult to isolate. GLC retention data provide rapid, tentative identification of many compounds. Combination of GLC with time-of-flight mass spectrometer analysis separates and identifies substances in fractions of a part per million including compounds whose separate existences are transitory. Spectral data other than mass spectra are useful to establish chemical structure of difficult compounds. Milligram quantities of pure material must be isolated to obtain these spectra and these new highly efficient columns will help to prepare such samples as well as the samples of individual volatile components of fruits needed for taste evaluation.

Studies were initiated to develop tissue cultures from flavor-rich grape varieties. These cultures grown in the bland juice of Thompson seedless grapes appear on the basis of preliminary investigations to give some of their own flavors to the Thompson juice. Controlled fermentation of tartaric acid with the "noble rot" mold also improves flavor of Thompson juice.

6. Pioneering Research: Ethylene Metabolism in Fruit. The enzymes involved in the metabolic fate of ethylene in fruit under conditions wherein ethylene hastens the post-harvest maturation continue to be investigated, using ethylene labeled with C^{14} and with H^3. From a comparison of the amount of radioactivity and the labeling pattern of the toluene and benzene arising from the metabolism of the two labeled ethylenes, it could be postulated that during the metabolism of ethylene a considerable portion of the hydrogen is removed from ethylene by some dehydrogenation process, and this hydrogen proceeds along a pathway different from that followed by the carbon as illustrated by the labeling of toluene. Part of the hydrogen and carbon are metabolized along the same pathway as illustrated by the labeling of benzene. This postulate suggests that acetylene, which produces some of the same effects on plants as does ethylene, may yield some of the same metabolites as does ethylene-C^{14}. This was found to be true. Acetylene-C^{14} was metabolized to an extent ten times as great as was ethylene-C^{14}. A large proportion of the metabolized acetylene was found in benzene. The isolation and identification of other volatile and non-volatile metabolites of labeled ethylene are continuing in order to obtain additional information about the enzymes involved in the maturation of fruit with the ultimate goal of controlling maturation prior to processing.

Protein Synthesizing Enzymes. The first step in protein synthesis is the activation of the individual amino acids and their transfer to ribonucleic acid (RNA). Each amino acid presumably has a specific activating enzyme and a specific RNA to which it is attached. The structure and conformation of the RNA molecule required for acceptance of an amino acid is under study. The secondary structure of RNA is disrupted by the presence of relatively large amounts of purines. It has now been established that purines are without effect on the activation reaction. However, the effect of disruption on the transfer reaction remains to be established.

Biosynthesis of Diosgenin. The role of steroids in plant tissue, particularly as related to enzyme action and the control thereof, is under investigation. One of the several steroidal sapogenins which occur in certain plant tissue is diosgenin. The following sequence of reactions has now been demonstrated by administration of radioactive precursors to plants and isolation of the radioactive products by thin-layer chromatography: Acetate ⟶ mevalonate ⟶ squalene ⟶ cholesterol ⟶ cryptogenin ⟶ diosgenin.

Desoxyribonucleic Acid Polymerase. The control of enzyme action and physical properties of plant tissue is through the genetic information contained in the desoxyribonucleic acid (DNA). In nature the DNA is associated with histones (basic proteins) whose presence presumably regulate the synthesis of enzymes. In order to obtain more information in this deep-seated question of genetic control of enzyme action, the influence of the presence of histones on DNA replication in vitro was investigated. It has been found that pea DNA complexed to histone can function as a template for DNA replication, but only to a limited extent as compared with uncomplexed DNA. Further pioneering research on the possible control of enzyme action through such a basic approach will be pursued.

Chemical Origin of Plant Structural Tissue. The chemistry and biochemistry of formation of plant cell walls is under investigation because of the obvious importance of cell walls to such characteristics as texture, turgor, and cell elongation. The reproducible tissue culture of tobacco cells as a model system is used in this investigation. The proteins of these cells can be separated into protoplasmic, cell wall-extracted and cell wall-residual fractions, each with its different amino acid composition, particularly with respect to the amino acids, proline and hydroxyproline. The protein of the cytoplasm has a high biochemical turn-over whereas that of the cell wall is very low, suggesting that the former is converted to the latter. Characterization by electron microscopy autoradiography of the cell wall proteins and their role in cell wall structure and properties will continue with particular emphasis on their synthesis and deposition in the cell wall.

B. New and Improved Food Products and Processing Technology

1. Dried Fruit Products. Research on new and improved dried fruit products continues, supported in part by the Dried Fruit Industry Research Advisory Committee, which provides the salary of one of the scientists assigned to this work. A process for making a non-setting raisin paste was reported earlier. Raisin paste can be used in many bakery formulations, but heretofore tended to set in a hard lump so the bakers had to prepare it just before use. The process was improved by use of a drum dryer. An extremely short-time heat treatment retarded setting of raisin paste. Ground raisins in contact with the surface of a drum dryer for as short a time as 28 seconds with the drum heated to 190-200° F. remained soft and pliable. Only about 1% of the moisture was lost during the treatment.

Stability studies of dried fruit continued. Storage life of conventionally dried apples was determined to be about 4 months at 90° F., 11 months at 70°, and well over a year at 50°. The darkening rate of apples was approximately 30 times greater at 90° than at 50°. When sulfured dried fruits with different levels of sulfur dioxide are packed together, the protective chemical migrates from the fruit with the higher sulfur dioxide level to the others. For example, prunes which are not normally sulfured were found to contain as much as 900 p.p.m. SO_2 due to migration from cut fruits in the same package.

High quality dehydrated Comice pears (a winter variety) were produced by the DBD method (partial drying--steam blanching--finish drying). Commercial interest in the process exists for marketing in a high-quality specialty market area.

2. Fruit Dehydration. A novel dehydration procedure for pieces of fruit was developed. Fruit pieces were dried in a matrix of various sugars, starches, or celluloses. These matrices provide a medium for heat and moisture transfer and have been used over a range of conditions including vacuum drying, fluidized bed drying, and as pre-treatments before stationary

hot air drying. Color and texture of dehydrated or partially dehydrated fruit was best with a sucrose matrix. Fruit slices were prepared for dehydro-freezing with crystalline sucrose. The partially dehydrated product had color, texture, and flavor equivalent to fresh fruit. These exploratory studies, in which moisture is withdrawn from the fruit by the difference in osmotic pressures between the material within the tissue cells and the dry matrix, open the door to a wide range of new possibilities in the dehydration of foods. Apple wedges or slices, or other fruits such as whole berries, were stored with an equal weight of sugar overnight at room temperature or for about 2 hours at 120° F., preferably after a dip in a weak sulfur dioxide solution. Treatment reduced the fruit to about 50% of its original weight and the sugar has become a heavy syrup. The fruit was separated from the syrup and dried in hot air at 160-180° F. to a moisture content as low as 3-4%, if desired. The dried product had excellent color and flavor. The first step in which water was removed by osmosis appeared to set the structure so that the remaining water was easily removed to a very low moisture content. It is a slow process to prepare dried apple slices below 20% by conventional methods because the structure collapses in the early stages of dehydration and resists transfer of moisture. Larger quantities of osmotic dried apple slices are being prepared on pilot scale for chemical and taste evaluation. The improved quality apparent in these dried apple slices makes them appear of potential value in domestic markets even though dried apple slices as they now exist have very limited demand. Fruit dried in this way needs little sulfur dioxide as a preservative and retains good flavor. The heavy syrup produced by the withdrawal of moisture from the fruit has a very good flavor of the fruit processed. The growing use of fruit-flavored syrups for breakfast use may offer a valuable market for this byproduct of the osmotic dehydration process. As an alternative process, the initial drying can take place in heavy syrup instead of a sugar matrix and the syrup can be concentrated and recycled.

3. Texture of Processed Fruit. Serious outbreaks of brined cherry softening have plagued the Northwest cherry industry from time to time in the past several years. Cooperative studies have been conducted with the Oregon and Washington State Agricultural Experiment Stations and with interested processors and growers to determine the cause and develop controls. After several years of study, all the causes of deterioration of brined cherries are still unknown, although activity of pectic enzymes has been frequently detected and is almost certainly involved. The sources of the pectinolytic enzymes may be several. However, procedures aimed at inactivating enzymes and firming the pectic materials in brined cherries were developed that gave good protection against deterioration. Heating cherries to 160° F. either before brining or in the brine prevented enzymic softening. High-calcium brines also protected against softening. Commercial processors are interested in the possible re-use of brine in cherry preservation for economy and to avoid problems of waste disposal. Brine that was used three seasons in a commercial plant produced satisfactory firm cherries except in tanks of brine containing active pectinolytic enzymes. However, the firm cherries, after being processed into maraschino cherries, developed undesirable brownish red color

in prolonged storage of the finished product. The color degradation has not yet been unequivocally linked to the re-use of the brine, because a new dye formula was involved in the maraschino process.

4. Grape Juice and Grape Products. Research on viniferous grapes is continuing to seek new products that will enlarge markets for wine grapes grown beyond the needs of current markets. Principal attention is directed towards Thompson seedless grapes which are the major surplus burden. Diethylpyrocarbonate was tested as a chemical sterilant to avoid cooked flavor developed by pasteurization to allow pure culture fermentation improvement of Thompson seedless grape juice. It was possible to grow pure submerged cultures of Botrytis cinerea (noble rot) which metabolizes tartrate in the juice and develops improved flavor. The conversion of the non-metabolizable tartaric acid, would make the grape juice more desirable in food products such as baby foods. Cultivation of Botrytis cinerea (which grows on grapes fortuitously in some seasons in some parts of Europe to make very high-quality wines), in non-alcoholic grape juice improved flavor substantially as judged informally by scientists working on the project. We devised a scrubbing apparatus for filterless sterilization of the input air, which acts as an agitator and source of oxygen in submerged culture fermentations, and also conducted chemical studies of the acid changes in aldehyde formations.

Osmotic concentration of grape juice by dialysis demonstrated the possibility of reaching 64° Brix in three hours. Several membranes were tested in this new process. Experiments with common commercial cellophane indicated the feasibility of concentrating the juice by use of salt as the hypertonic solute. No acidification was found necessary if cellophane was the membrane. This simplified the subsequent concentration of hypertonic medium which is necessary as it approaches equilibrium with the grape juice.

After seven months' storage at 0° F. commercial Concord grape flavor concentrate lost more methyl anthranilate than samples stored for the same period at 32° and 60° F. In this storage time losses in total volatile esters remained negligible at both 0° and 32° F. and amounted to only 10% at 60° F. For purposes of economic efficiency commercial production of grape juice concentrate requires that the product be stored in bulk as it is produced during and soon after the harvest season. It is not known what the optimum storage conditions are for the concentrate nor is it known whether it is feasible or desirable to remove volatile esters and store them separately from the concentrate until they are finally packed. Preliminary laboratory tests are being conducted on grape and apple concentrates.

5. Processing Quality of Varieties of Northwest Fruit and Berries. Fruit and berry selections are evaluated in cooperative research with the Washington State Experiment Station. The choice of varieties of strawberries and raspberries for processing in the Pacific Northwest depends very much upon these cooperative studies. In 1963, 92% of the strawberry crop was from new varieties developed by the Experiment Station and tested for processability by Department scientists. Raspberries, blackberries, blueberries, apricots, and cherries were evaluated during the past year.

Fifteen selections of strawberry crosses are now left from 10,000 seedlings planted in 1960. The detailed studies that will now be possible on the reduced number of seedlings remaining should provide additional varieties that will be superior in both production and quality for release to commercial growers.

The current leading commercial raspberry varieties accounting for nearly 90% of all raspberries grown in Washington, were tested extensively over several years at the Puyallup station for processing suitability before release to the trade. Two apricot varieties and two cherry varieties of early maturity, which were recently released by the State Station, also were tested. So large a proportion of the berries and fruit grown in the Pacific Northwest is processed that new varieties must adapt to processing in order to succeed.

6. Improved Fruit Juices and Fruit Juice Processes. The initiation of a pack of full-flavored concentrated frozen apple juice was a major development in apple processing in the Pacific Northwest. The commercial operation is based on developments of the Western and Eastern Utilization Research and Development Divisions and the extension of laboratory and pilot plant data to a successful commercial venture was carried out with technical assistance from the two Divisions. Specific technical problems were anticipated from the small but important amounts of starch in apple juice. Enzyme treatment was found essential to produce stable frozen concentrated apple juice during the early season when appreciable starch is present. An iodine test for starch in apple juice as the juice enters the concentrator was found to be a useful index of final stability. One-year storage tests on frozen concentrated apple juice, which had received no treatment to remove pectin, showed no evidence of pectin gel formation during frozen storage.

Efficiency of operation and maximum recovery of juice from apples depends upon maintaining a minimum of insoluble solids in the pressed raw apple juice. It was found essential that the apple pulp not be manipulated more than absolutely necessary between milling and pressing in a rack and cloth press in order to maintain low insoluble solids content.

An improved apple juicing system was conceived and tested on recently harvested and stored apples two months and six months after harvest. The conventional rack and cloth press was replaced by a sanitary basket centrifuge and a vertical screw press system using standard pieces of food processing equipment. Tests were run in the Pacific Northwest in October, December, and April. Gross yields of apple juice were 10% greater than for rack- and cloth-pressing; pectinol lees were about 10% lower.

7. Stabilizing Shelled Nuts. The attractive appearance and convenience of shelled nuts is increasing the proportion of nuts sold in this form. Once the shells are removed, kernels tend to darken and turn rancid rather quickly. An investigation of the deterioration of shelled nuts is supported

in part by the Diamond Walnut Growers, Inc., which supplies the salary of one chemist assigned to the Pasadena, California Laboratory. Detailed analyses of fresh and rancid walnut kernels revealed that changes in the nitrogenous constituents and volatile organic compounds occur during the development of rancidity. One unknown compound in the basic amino acid fraction was destroyed and a number of new compounds were formed during rancidity development, suggesting interactions between the nitrogenous constituents and lipid oxidation products. The volatile products of lipid oxidation will now be studied to determine their chemical nature and interaction with nitrogenous and other compounds during the development of rancidity.

Research on the stability of fresh and roasted Macadamia nuts was concluded under a contract with the University of Hawaii. Three varieties of Macadamia nuts were shelled, roasted and evaluated for stabilities at three moisture levels over a reasonable temperature range, protected and unprotected from light. Low-temperature storage in the dark increased stability. For samples stored at 1%, 2.5% and 4% moisture, rancidity increased as moisture decreased.

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AREA NO. 7. POTATOES--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, is becoming more and more dependent upon the development of new and improved processed products to maintain markets and to avoid recurring economic disasters. Crop perishability, supply fluctuations, and the inelasticity of demand, result in wide swings in price with even slight surpluses. In producing areas having a substantial processing industry, depressive lows are moderated by advance contracting by processors prior to harvest. However, in many important potato growing areas processing has not yet developed, and vulnerability not only still exists, but is exaggerated by the growing competition of processed potato and other competing food products. A continuing improvement in processed potato products is clearly required if processing is to expand fast enough to offset the progressive decline in use of fresh potatoes.

To improve the quality of processed potatoes, ways must be found to eliminate the stale, earthy, rancid, green, and warmed-over flavors that are sometimes encountered in potato products, including dehydrated mashed potatoes, dehydrated diced potatoes, frozen French fries, frozen patties, and potato chips. Equally important, methods must be devised to retain the desirable natural flavor of the freshly cooked potato in the processed product. Recently developed research methods offer an opportunity to isolate and identify the constituents responsible for the natural flavors and the off-flavors, to develop rapid and sensitive analytical methods for their measurement, and to determine the raw material factors controlling formation of the various desirable and undesirable constituents in the fresh potato. Further improvement in the texture of potato products is also needed. Fundamental histological and chemical investigations could be used to determine the causes of differences in the texture of potatoes, as a basis for developing improved processing methods. Enzymes play a great part in the entire compositional pattern of the potato, not only the constituents responsible for flavor, off-flavor, color, and texture, but also those responsible for disorders such as black spot. Black spot causes severe losses both to those who market potatoes fresh, and to those who process potatoes, because trimming costs are sharply increased and yields reduced. Increased knowledge of enzymes is needed as a basis for solution of the black spot and similar problems, to increase use of potatoes by reducing costs, and to improve quality of both fresh and processed potatoes.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, basic and applied research on potato products is conducted at the Division headquarters at Albany, California, and by grant funds under P.L. 480 in England and

Sweden. The chemistry of potato flavor and the compounds involved in deterioration of potato products are studied to provide a basis for new and improved potato processes and products. Histochemical studies are conducted to elucidate factors involved in the texture of potato products. Basic investigations on the enzyme systems involved in potato product discoloration and the mechanism of rancidity development are in progress.

The Federal program of research in this area totals 6.3 professional man-years. Of this number, 3.4 are assigned to chemical composition related to flavor, color, and texture of potato products and 2.9 to technological and engineering research on new products and processing methods. In addition, the Division sponsors two research grants under P.L. 480 on basic studies.

PROGRAM OF STATE EXPERIMENT STATIONS

For many years State stations have conducted research on problems related to potato utilization. Studies designed to evaluate, by biochemical and physical means, the effects of such production variables as fertilization, variety, location of production, harvest maturity, handling and storage on composition and use of potatoes are continuing.

The trend toward increased processing has added emphasis to work designed to evaluate processing quality of potatoes. A prime problem associated with the rapid growth of potato processing is maintaining quality of tubers during extended storage. Production variables and storage conditions affect potato composition. Mechanical harvesting systems frequently affect potato quality and composition. Yields; total solids; suitability for french frying, chipping, boiling, mashing and baking; and sugar content are routinely determined. More thorough chemical composition studies are carried out to determine content of phenolic acids, nitrogen, nitrogen-free organic acids, amino acids and reducing sugars as a means for better understanding utilization problems.

Interest in problems of discoloration and the role of nitrogenous compounds in enzymatic blackening leads to studies on the color reactions of polyphenol oxidase plus chlorogenic acid and/or caffeic acid with amino acids found in potatoes. Recently work is being initiated to determine the relationship of lipid content, both total and fatty acid composition, to discoloration of potatoes. The relationship of chemical and physical properties of potatoes to sloughing is also being studied.

New techniques for conduct of flavor investigations are applied to potatoes and potato products. For example, gas chromatographic techniques are used to study the volatile compounds of potato chips as a part of the attempt to isolate and identify the chemical compounds responsible for the flavor and aroma of chips. This work is tied in with efforts designed to find ways to maintain or extend fresh potato flavor and improve chipping quality or shelf-life.

Product research includes work designed to determine physical factors affecting the color or lack of brightness of reconstituted dehydrated potatoes. The relationship of specific gravity and starch content to the texture of frozen french fried potatoes is being investigated. Another study deals with the effect of pre-preparation, preparation, and post-preparation techniques on the quality, yield and cost of potatoes prepared in quantity.

The potato utilization research program also includes investigations designed to work out new or improved uses for sweet potatoes. New methods of processing grades currently not acceptable by the fresh market are sought. Suitability for canning, freezing and dehydration is determined. Means of using sweet potatoes in new products or other food products are investigated.

The total number of professional man-years devoted to potato utilization research is 7.7.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition Related to Flavor, Color, and Texture of Potato Products

1. Flavor Stability. The inclination of dehydrated potato products to oxidative deterioration is a serious commercial problem. Most unstable food products keep better under refrigeration. However, at least three large commercial trials at stabilizing potato granules or flakes by refrigeration have failed, apparently because temperatures as low as 0° did not prevent rancidification. Antioxidants are now used in most commercial dehydrated potatoes. Such use of antioxidants was guaranteed to the public domain by a Department Public Service Patent granted about 10 years ago.

Over the past several years techniques were developed to use gas-liquid chromatography to follow oxidative changes in dehydrated potatoes. An intensive stability study is in progress using gas-liquid chromatography and taste panels to measure oxidative changes in potato granules of three moisture levels at five storage temperatures in atmospheres of air and of nitrogen. Oxidative rancidity, as measured both chemically and organoleptically appears faster (1) at higher temperatures, (2) at lower moisture contents, and (3) in the air package atmosphere rather than in nitrogen. For comparison, samples were packed in an atmosphere of oxygen, where oxidation proceeded so rapidly that no realistic stability tests were possible. Under these conditions temperature had little effect. Even at -20° F. oxidation was very apparent in three months and proceeded only at a slightly lower rate than replicate samples stored at 70° F. Because the oxidation of dehydrated potatoes is believed to be closely tied to the lipid components, a comprehensive basic investigation of potato lipids is required and will be brought into the potato research program in the future.

Related basic research on the autoxidation of fats in systems that exist in dehydrated vegetables is being conducted under P.L. 480 grant at the Swedish

Institute for Food Preservation Research, Gothenburg, Sweden. The oxidation measurements are based on oxygen consumption from the headspace over fatty acid solutions used as model systems. The conventional Warburg apparatus was not suitable for studies at low oxygen pressures so a modified system was developed by which consumed oxygen from the headspace is automatically replaced by electrolysis and the rate of electrolysis is recorded. Satisfactory results are being obtained and measurements are being conducted with linoleic acid emulsions and linoleate solutions.

2. Enzymic Browning. Basic studies of the enzymic browning of potatoes are in progress at the Low Temperature Research Station in Cambridge, England, supported by P.L. 480 grant funds. The research is aimed at determining the nature, distribution, and mode of action of enzymes responsible for discoloration of uncooked potatoes and potato products, and to determine the nature and distribution of the phenolic enzyme substrates and the pathways of their synthesis. Variety, climatic factors, and cultural practices were investigated to determine their effects on the enzymic browning of cut potatoes. The major rate determining factor of enzymic browning appeared to be the concentration of tyrosine in the tuber. The level of tyrosine in the potato was demonstrated as being influenced by genetic, climatic, and to a lesser extent, cultural factors. Of the climatic factors, rainfall or the moisture available to the plant appeared to be the most important. No direct correlation was found between the rate of browning and the activity of the phenolase enzyme or the amount of chlorogenic acid (a phenolic enzyme substrate widely found in plant material). In basic comparative studies, phenolases and phenolase substrates from plant material other than potatoes, are under investigation. Phenolase from the broad bean (Vicia faba) was shown to differ from potato phenolase mainly as it may be activated or inhibited by specific reagents. Potato enzyme was active over a wider range of substrates. Sodium chloride and other salts inhibited the potato enzyme most effectively at a different pH than the enzyme of the broad bean.

3. The Role of Sulfur Dioxide in Dehydrated Vegetables. A basic investigation of the chemical mechanisms involved in the protective action of sulfur dioxide against browning of dehydrated vegetables was concluded at the Covent Garden Laboratory in London, England, where it had been supported by P.L. 480 funds. This highly successful investigation resulted in 10 scientific publications and led to the following broad conclusions:

(1) For non-enzymic browning to occur rapidly, the presence of a sugar is less important than the presence of an amino acid or similar type of nitrogen-containing compound;

(2) A common class of intermediates in several types of browning reactions originated from widely different compounds. These highly reactive intermediates (alpha-beta-unsaturated aldehydes) are frequently a point of departure for further reactions with nitrogen compounds, leading to brown polymers;

(3) In model chemical systems and to some extent in vegetable systems, the mode of action of several inorganic ions in modifying sugar-amino acid browning rates can be related to the effect of these ions on the initial reactivity of the sugar component;

(4) In retarding non-enzymic browning bisulfites are involved in more than one type of reaction;

(5) Two modes of bisulfite action were identified during this work that had not previously been reported. First, bisulfite lowered the initial reactivity of the sugar by affecting the relative concentrations of the isomeric forms of the sugar molecule. Second, bisulfite reduced all those browning reactions that involve alpha-beta-unsaturated aldehydes as intermediates.

(6) Indications exist that reactive intermediates can form various cyclic structures which may be incorporated in brown polymers. The polymers so formed have groups which give them ion exchange properties for metallic ions.

B. Technological and Engineering Research on New Products and Processing Methods

1. Frozen French Fried Potatoes. Frozen potatoes make up nearly a third of all the frozen vegetables produced in the United States. Well over a billion pounds of potatoes are utilized for frozen foods each year. About 85% of the frozen potato products are frozen french fries and of these nearly three-quarters are packaged for institutional sale. Although it is not recommended, small restaurants with inadequate freezer space commonly hold several days' supply of frozen french fried potatoes in the refrigerator at above freezing temperatures where deterioration in flavor and texture readily occur. The stability of oil-blanched frozen french fried potatoes at a storage temperature of 55° F. was investigated. A taste panel gave significantly lower scores in flavor, texture, and color to products that had been finish-fried in deep fat after three days' storage at 55° F. than to french fries that had been held at 0° F. Gas-liquid chromatography showed an increase in the hexanal peak with increased storage time. Microbial counts increased by seven orders of magnitude over five days. Stability studies will continue at temperatures intermediate between the 55° F. and freezing. It is probably too expensive for some small restaurant operations to keep french fried potatoes frozen. The results of this investigation will provide information on safe limits of temperature and time for the restaurant handling of frozen potato products.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition Related to Flavor, Color, and Texture of Potato Products

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Technological and Engineering Research on New Products and Processing Methods

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1/ Research supported by P.L. 480 funds.

AREA NO. 8. VEGETABLES--PROCESSING AND PRODUCTS--WESTERN LABORATORY

Problem. Vegetable crops, in general, are perishable and seasonal and thus, are subject to supply and price fluctuations to the disadvantage of the agricultural economy. In order to expand markets and stabilize prices, new and improved processed products are needed that will be more desirable to the domestic and foreign consumer from the standpoint of quality, convenience, stability, nutritive value, safety, and cost. The quality of processed vegetables and the economy of their processing have not improved rapidly enough to increase or even maintain the relative position of vegetables in the American diet, or to increase substantially their contribution to the export trade. The consumption of dry beans and certain other vegetables is limited by the fact that they cause flatulence.

New easy-to-prepare vegetable products are needed, particularly from such commodities as dry beans and peas, which now require hours to prepare. The severe heating required to sterilize low-acid foods, which include most vegetables, seriously impairs the quality of canned products. The stability of all kinds of processed vegetables needs to be improved so that quality and nutritive value will be better preserved during storage and distribution. The safety and effectiveness of new chemical additives, needed to improve the quality and stability of processed vegetables, must be established. Better methods of removing residues of agricultural chemicals from vegetables for processing are urgently needed, as are procedures for decontaminating vegetables exposed to radioactive fallout. Of vital importance is research to reduce the costs of processing in order that the farmer may receive a larger share of the consumer's dollar.

Applied research on these practical problems must be supported by a strong program of basic research on the chemical constituents of vegetables responsible for flavor, color, and texture; on the reactions these compounds undergo before, during, and after processing; on constituents having biological activity; on the microscopic structure of vegetables and vegetable products; and on the micro-organisms which cause spoilage or loss of quality in these products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic research on vegetables and the application of science to new and improved products and processes is conducted at the Division headquarters at Albany, California, in field stations at Pasadena, California and Puyallup, Washington; by contract at Urbana, Illinois, East Lansing, Michigan, and Davis and Berkeley, California; by a grant at Urbana, Illinois, and by grants under P.L. 480 in Cambridge and Chipping-Campden, England. Fundamental studies are conducted on the chemistry of vegetable flavor and

vegetable pigments, the mechanism of heat resistance in bacterial spores, the composition of dry beans as related to cooking quality and flatulence-producing characteristics, the factors affecting deterioration of dehydrated vegetables, and the microbiology of raw vegetables for processing. Applied research is conducted to develop new and improved products to increase the utilization of vegetables including new, high quality concentrated and dehydrated products and products of improved convenience of use, processes for producing these, selection of improved processing varieties, and methods for removing radiactive fallout.

The Federal program of research in this area totals 38.6 professional man-years, including three scientists whose salaries are provided by the California Lima Bean Advisory Board operating under a State Marketing Order, the United States Brewers Association, and the National Association of Frozen Food Packers; and five contracts and grants equivalent to approximately 2.5 professional man-years per year. Of the total, 20.1 are assigned to investigations on chemical composition and physical properties and 18.5 to new and improved food products and processing technology. In addition, the Division sponsors three grants under Public Law 480 on basic research.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations have a continuing long-term research effort devoted to vegetable processing and products. Research on the adaptability and evaluation of vegetable varieties for processing is a standard service to plant breeding programs. Before introduction of new varieties, processing yields and processing characteristics are determined. This type of research extends to consideration of the effects of various production variables on processed product quality. Effects of maturity at harvest, mechanical harvesting, fresh product characteristics, post-harvesting handling and storage are examples of problems under study. The degree of correlation or association between color and flavor in fresh and in processed items is always of major concern.

Chemical composition and physical properties are also related to product acceptance and quality. This research ranges from standard composition studies to basic studies of special components. For example, the non-cellulosic constituents of plant cell walls are being investigated. The nitrogen compounds in mushrooms are determined and evaluated as a function of previous history of growth, handling and processing of mushrooms. A continuing analysis of the biochemical changes that occur in vegetables at the different stages of maturity is made. Other studies deal with determination of the antioxidant properties of chile and the antioxidant effects exerted in the various kinds of meats. Estimates of the metal complexes of chlorophyll derivatives in processed foods and their effect on the color of processed foods are made through processing experiments conducted under controlled conditions of metal contamination in the laboratory or in commercial processing plants. The role of enzymes in chemical and physical changes in processed foods is studied through use of purified enzyme systems, substrates and reaction products.

In order to obtain a better understanding of the reasons for changes in flavors during processing and storage and for the development of off-flavors, a comprehensive program on flavors in processed foods is in progress. Heat-induced flavors; lipids in flavor, bitter flavor of carrots, and natural fresh flavors of vegetables are all under study.

Microbiological research extends from study of the natural flora found on fresh vegetables to studies of contaminants found in commercially processed foods. Methods for microbiological examination of foods are being developed. Physiological, morphological, and nutritional variation among important organisms are determined to facilitate control of the organism or essential understanding of the role of the organism in desirable or useful applications. Bacterial endospores receive much study. It is hoped that this work will lead to the improvement of present methods of sterilization and food preservation. The radioresistance of bacterial endospores and use of combined antibiotics and heat are carefully researched to provide information of use in developing new and improved procedures for canning vegetables. Food poisoning organisms are the object of continuing interest. The incidence of spoilage organisms, survival patterns, and means of control are being investigated. Studies on the effect of carbon dioxide inhibition of microbial growth are in progress. The microbiology of processed foods, for example--dehydrated foods, is another area of research activity.

New and improved vegetable processing technology is sought in studies of freeze-drying, brining, canning, dehydration, fermentation, hydro-cooling and controlled atmosphere methods. Basic studies deal with new techniques of soaking and preparation, enzyme inactivation and regeneration, fluid flow and heat transfer problems. Special attention is being given to development of high-temperature, short-time methods and the advantages of low-temperature handling of sterilized foods. A comprehensive study of the effects of controlled or modified atmosphere on the biochemical, physical and general quality characteristics of various vegetable products is in progress.

New product research with vegetables is directed toward development of "quick cooking" peas and beans; beet chips; Puerto Rican style soups; snack items; and new sauerkraut products. Methods of processing, product characteristics and storage stability are determined. Some pilot plant research is done, but basic principles relating to composition, quality and functional properties are emphasized. Product characteristics such as sweetness, concentration of individual sugars, rheological properties, softness, water absorption, color and pigment are related to organoleptic properties and consumer acceptability.

The total station scientific research effort devoted to vegetable processing and products is 46.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Vegetable Flavor Components. Basic research on vegetable flavor is continuing through the study of the chemistry of volatile components using highly sensitive laboratory equipment and through evaluation of the olfactory response to individual compounds and groups of isolated compounds from vegetables. Panels of judges are used to measure the most dilute concentration of pure compounds that can be detected. Threshold concentrations data are beginning to show how odor strength is related to molecular structure. For example, the odor strength of a series of straight-chain saturated and alpha-beta unsaturated aldehydes progressively increases as the chain length increases from five to ten carbon atoms. An important question was settled by the clear demonstration that compounds of these types when present in undetectably small concentrations can add with each other to contribute a detectable odor to a mixture. Such results support the contention, which has frequently been stated but heretofore not unequivocally proved, that very minute amounts of substances in foods can contribute to the total flavor response even though they cannot be individually detected subjectively and perhaps exist only as traces in the most refined gas-liquid chromatographic separation of volatile materials. A Department scientist has presented the basic concept that the molecular shape of a chemical compound dictates its odor. He described how seven basic odors correspond to five specifically shaped receptor sites on the olfactory nerve endings and two other as yet imperfectly defined receptors. This stereochemical theory of olfaction is being subjected to critical research evaluation. Linear dimensions, cross sectional areas, and volumes of molecular models were measured and the goodness-of-fit to the postulated olfactory receptor sites estimated. Twenty-one chemicals having fairly rigid molecules and a camphoraceous odor were tested and although very diverse chemically, they were found to be nearly identical in size and shape.

Research is conducted on the composition of peas in relation to canned pea flavor. This work has been supported in part by the National Canners Association, which provided the salary of a chemist assigned half time to the project. Investigations of compositional differences among fresh, frozen, and canned peas revealed much higher levels of volatile compounds in fresh peas than in the two processed products. The appearance of propionaldehyde and hexanal in the canned pea samples suggested their formation during the heat sterilization process. Organoleptic evaluations were conducted to ascertain the importance of various compounds isolated from peas and processed peas. Dimethylsulfide has a low olfactory threshold and appears to be a major contributor to the flavor of canned peas as well as other vegetable products. When methionine was heated in the presence of pectin, dimethylsulfide was generated. Methionine, an essential amino acid in human diet, and pectin are both present in peas and many other common foods. Panels rated peas to which the non-caloric sweetener, hexamic acid, had been added above peas with no additives. If its addition to canned

peas can be demonstrated further to improve flavor, the regulatory agencies should offer no objection to its addition on the basis of public safety.

Research on the volatile components of tomatoes and tomato products was initiated. Substantial quantities of methylsulfide were found to be produced during heating and cooking of tomatoes. Processed tomatoes are always heat-treated and the usual standard of tomato flavor is high-quality, single-strength tomato juice rather than the juice expressed from a fresh tomato. A large quantity of fresh tomatoes was processed in the laboratory to collect volatile components for compositional studies. Twenty-three alcohol and carbonyl compounds have already been identified but they represent only a small fraction of the total number of components present. The large quantities of free sulfur found in the volatile concentrate fraction suggest the presence of very reactive sulfur-containing precursors. The effect of heating on tomato juice was tested in two ways. Odor thresholds were determined and the products were analyzed for methyl sulfide. Presumptive evidence indicated that the aroma intensity of a tomato juice is related to its methyl sulfide content. This relationship will be more thoroughly investigated in the future.

Compositional research on the essential oils extracted from hops is supported by the United States Brewers' Association which has provided the salaries of two chemists. The long-range purpose of this project is (1) to isolate and characterize volatile components that may contribute to the flavor of hops and (2) to study the chemistry of such components. Organoleptic evaluations of various fractions of hop oil and of recombinations of individual volatile constituents closely simulate the original hop oil flavor. Continued use of the capillary gas-chromatography-mass-spectral method of analysis has identified components of the oxygenated fractions of hop oil. Nineteen previously unsuspected compounds have been added to the growing list of known volatile hop oil components. One of the newly identified components is a thio-ester. Thio-esters have not been found widely in natural products. Odor thresholds of the isolated components of hop oil are being determined. About 16 components have thresholds ranging from 0.8 parts per billion to 250 parts per billion. Such identification of the relative contribution of individual components to the aroma of hop oil should provide the basic tools for controlling quality and making more uniform and better hop products.

2. Nature of the Heat Resistance of Bacterial Spores. The extreme resistance of bacterial spores to heat demands processing that degrades flavor, color, texture, and nutritional quality of canned vegetables. Basic investigations of the nature of heat resistance exhibited by spores have continued and have already led to a process which in the laboratory allowed a 50% reduction in heat treatment to sterilize a vegetable product containing large inoculations of spores of B. stearothermophilus, B. subtilis, and Clostridium #3679 (these organisms are frequently used for testing adequacy of heat processes).

Improved laboratory techniques were developed to separate various spore samples and to prepare them for detailed chemical investigation. A new controlled dry rupture procedure is highly reproducible and permits a preselection of the degree of rupture. This process separated spore coats without chemical changes that would be induced by heat or moisture. Ultraviolet absorption spectra of dry spores and of separated spore coats revealed the presence of calcium or magnesium chelates of dipicolinic acid in five different species. The relatively large quantities of dipicolinic acid and calcium provoked considerable speculation. The findings reported here are good evidence that calcium and dipicolinic acid chelate together in the bacterial spore. Spore separation by density gradient was advanced by the synthesis of a series of new, water-soluble, neutral, lead chelates which provide solutions of variable, high density but low ionic strength. The technique will aid in preparative separation of spores and studies on the heterogeneity of spore populations. The dry rupture and the density gradient separation procedures should apply widely in biological research beyond the study of bacterial spores.

Studies on heat adaptation of bacterial spores led to the observation that resistance to heat can be irreversibly reduced by briefly creating acid conditions around the spores. Low-acid vegetables can be treated briefly by addition of acid and then neutralized prior to canning. The acid treatment cut in half the heating time necessary to sterilize canned food in the laboratory. To guarantee the general use of this development, application was filed for a Public Service Patent covering the procedure.

3. Composition of Dry Beans. A systematic approach to determining the factor or factors in beans responsible for human flatulence is conducted by selective extractions followed by dialysis and ion exchange separations of bean components and measurement of the response to separated fractions when ingested. By these procedures the major part of the active principle was cornered in a fraction making up only a few percent of the total solids of the original beans. The active component was found to be soluble in both 60% and 85% aqueous ethanol and of molecular weight low enough so that the molecules can pass through a cellulose dialysis membrane. The fact that the flatulence principle could be separated from protein, led to a laboratory-scale process for preparing a flatulence-free protein concentrate from dry beans that should be readily adaptable to commercial processes.

By informal cooperative experiments, exploration of the possible mechanisms responsible for flatulence in humans was conducted at the Oak Knoll Naval Hospital in Oakland, California. Preliminary measurement of peristaltic activity using telemetry and a swallowed pressure-sensitive radio transmitter, indicated no difference in peristalsis after eating beans. Confirmation of this finding would require exploration of other possible mechanisms. Informal cooperation with the Department of Nutrition, University of California at Berkeley, has provided preliminary information on the effect of increased ventilation due to heavy exercise on the pattern of hydrogen elimination in human breath and flatus.

Closely coordinated with the studies at Albany, California, is supporting research conducted by contract at the University of Illinois in Urbana. The Illinois workers tried to develop an electronic device for recording the volume of flatus passed by a human subject while fully ambulatory but technical difficulties forced abandonment of this approach. A very satisfactory alternative procedure was developed in which a rectal catheter connected to a soft plastic bag was used. Using a plastic bag to collect flatus rather than the gas-trapping train used for in-house work allows the subject greater freedom to conduct normal activities without interference. However, the periodic measurements by weighing the trap of the gas train and periodic measurements of composition provide detailed data very useful in interpreting experimental effects. For different specific investigations each method will be advantageous.

4. Cookability of Beans and Peas. Compositional studies, supported in part by the California Lima Bean Advisory Board, which provides the salaries of two scientists, are directed toward understanding the effect on cooking of qualitative and quantitative differences in the proteins of large Lima beans. Electrophoretic separation of individual protein components from dry and immature beans and from beans at various stages of germination and cooking revealed significant differences in proteins. These results imply strongly that the cookability of dry beans is related to shifting protein composition or by intermolecular bonding of proteins.

A basic investigation of compositional factors related to cookability of dry peas was conducted at the Fruit and Vegetable Canning and Quick Freezing Research Association Laboratories at Chipping-Campden, England, supported by a grant under P.L. 480. It was completed in June 1964. The investigators showed that the influence of phytate ions on texture of cooked peas is very small, despite earlier opinions and supporting data that calcium phytate complexing influenced cookability. Analysis of subcellular fractions extracted from dried peas established the distribution of various elements in different tissues. In high-quality peas of good cooking characteristics the calcium ions are predominantly associated with proteins of the cotyledon, whereas in hard peas the calcium ions are in insoluble forms and are concentrated in the cell walls. Methods for measuring binding constants for calcium-protein complexes were investigated.

5. Pharmacological Investigations of Dry Beans. Components of dried beans are under pharmacological investigation to seek information relating to intestinal distress caused by ingestion of cooked dry beans. One procedure with laboratory animals has been to inject suspect compounds or extracts into contiguous ligated intestinal segments, each having the same arterial count. Two substances included in a 60% ethanol extract of cooked beans were shown by direct observation to have a significant effect on the rat's small intestine. A buffered solution of S-methyl-L-cysteine was injected into one segment and an equal volume of buffer into the other. An hour later the ligated segments were excised and weighed. The experiment was repeated enough times to provide statistically significant data, indicating

that S-methyl-L-cysteine caused an increase in intestinal segment weight due to mucous production. Authentic S-methyl cysteine and a dialysate from extracted cooked beans which was indicated by chromatographic procedures to contain S-methyl-L-cysteine, both caused contraction of a smooth muscle preparation. Histamine also caused such a contraction. An antihistaminic drug (Benadryl) inhibited the contraction of the smooth muscle preparation, leading to the conclusion that a histamine-releasing mechanism of action could be ascribed to S-methyl-cysteine.

6. Vegetable Pigments. Investigations on vegetable pigments are conducted to provide basic knowledge that can ultimately be applied to improve color quality of processed vegetable products. Modified analytical procedures for chlorophyll and chlorophyll degradation products were developed, including a resin-column technique to separate purified pheophytins a and b and a simplified thin-layer chromatographic technique for separation of pigment degradation products. Work on the development of new analytical procedures in this area was concluded and the methods so far developed are being applied to study the effects of processing variables on frozen, dehydrated and canned green vegetables.

Research on chemical changes in carotenoid pigments that result from processing vegetables and storing the processed products was concluded at the Low Temperature Research Station in Cambridge, England where it was supported with P.L. 480 funds. The investigation centered on the oxidative breakdown of beta-carotene to give the highly odorous compound, beta-ionone, and included studies on freeze-dried carrots and the chemistry of model systems. When foods are dehydrated by accelerated freeze-drying and subsequently stored in air, carotenoids oxidize, yielding volatile substances similar to those produced from the carotenoids themselves in chemical model systems. Many vegetables will lose vitamin A precursors by oxidation and plants high in carotenoids, such as carrots, will also lose color and develop off flavor. In addition, beta-carotene oxidation yielded large amounts of non-volatile hydroperoxide-containing polymers which may be toxic. These results point to a need to prevent the oxidation of carotenoids of food products such as by the use of antioxidants and also the need for more research in this area.

7. Histological Studies of Vegetable Tissue. Basic studies on the visual microscopic appearance of frozen vegetables are conducted with support from the National Association of Frozen Food Packers, which supplies the salary of one scientist. Structural damage caused by blanching and freezing of green beans is being investigated. The rate of ice crystal formation controls the size of ice crystals, which in turn controls the structural damage in frozen green beans. Moderate or slow freezing rates caused breakage of cell walls and separation of cells from each other. Very rapid freezing of green beans in liquid nitrogen greatly reduced damage to the frozen product and improved its texture after cooking.

Histological studies to acquire basic information on the mechanism of moisture movement and water-tissue relationships in vegetables were initiated

at the University of California at Davis under contract. Several vegetables, including onions, carrots, green beans, celery, green bell peppers, and mushrooms were used in preliminary work. For determining moisture content in the range between 10 and 70%, the Fischer titration method was found to be the most promising. The vegetables were dehydrated in progressive steps and judged by taste panels after reconstitution. Bell peppers could be dried to about 12% moisture with acceptable texture on reconsitution. Celery could be reduced in moisture only to 67% before it lost acceptable crispness. The other vegetables ranged between these two extremes. A better understanding of the mechanism of water movement through vegetable tissue during dehydration and rehydration in these different vegetables should yield information leading to improved processing methods and better products.

B. New and Improved Food Products and Processing Technology

1. Dehydrated Vegetables. Pilot-plant investigations of the foam-mat drying process were advanced far enough to study application of a standard process to various food products. Processors are most interested in drying tomato solids and pure lemon juice. The color and flavor of foam-mat dried tomato powder deteriorates if it is not protected from oxygen or if the moisture content is not below 3%. The processing conditions under which juice is extracted from tomatoes and concentrated also influences the stability of foam-mat dried powder prepared from the concentrate. For example, tomato powder made from high-quality, canned single-strength tomato juice had substantially greater stability than powder made from commercial tomato paste. Studies were initiated to determine if proper application of sulfur dioxide would prevent or substantially mitigate the changes induced by heat during concentration of tomato juice.

Studies on the critical problem of product burn-on of heat transfer surfaces are continuing with experiments on use of protein-splitting enzymes to reduce one source of burned material. Attention will be given to the rotating steam coil vacuum evaporators, which are increasingly being used in tomato concentration, and to control systems useful in reducing "burn-on."

Commercial processors of dehydrated vegetables believe their markets could be very much expanded if it were known how to process and package products so that they would better retain their initial flavor and color. In years past research has been conducted on the effects of processing and storage variables on the loss of color and flavor and the appearance of off-flavors in dehydrated vegetables, including the green vegetables. However, recent improvements in laboratory equipment and techniques offer considerable promise in providing a more detailed understanding of such changes that could lead to improved dehydrated vegetables. Research in this area is being redirected towards studies of the chlorophyll deterioration during processing and storage of green vegetables, utilizing some of the newer techniques of measuring chlorophyll components and products of chlorophyll degradation. Gas-liquid chromatography is being used to measure changes in

volatile components as a result of processing and storage effects. Organoleptic evaluations will be used to seek correlations with the objective measurements of product deterioration.

2. Dry Bean Products. The inconvenience of cooking dry beans and peas is widely believed to deter their utilization. Means for improving the convenience of cooking dry beans are being investigated, supported in part by the California Lima Bean Advisory Board. Processing large dry Lima beans to reduce cooking time was materially advanced. The processed beans retain the appearance of the usual dry Limas, have no detectable flavor difference, but can be cooked in 15 to 20 minutes without previous soaking. Dry beans vary widely and the time needed to soften the seed coats may differ from the time needed to soften the cotyledons. Reagents used in the process include specific edible chemicals for softening the seed coat and for softening the cotyledons. Occasional lots of dry beans require extremely long cooking. One lot of dry Lima beans that required 200 minutes or more to cook was altered by the new process to cook in less than 35 minutes. Stability studies indicate no serious regression in cooking characteristics during storage up to six months at room temperature when the moisture level remained between 8 and 12%. Studies at higher and lower moisture levels and at elevated temperatures have been initiated to determine the stability of the new quick-cooking Lima beans over a broader range.

We are also developing precooked legume powders. Such powders were prepared from a number of common varieties of dry beans, peas, and lentils, and each made into soup, snack dip, and other dishes to demonstrate versatility. A research contract was negotiated at Michigan State University to provide engineering data on a scale-up of the bean powder process as a preliminary step toward commercialization of the process.

Stability studies of unprocessed beans are also continuing. Large Lima, Sanilac, and Pinto beans at moisture contents of 10% or less did not change in cookability during two years' storage at 70° F. At higher moisture levels they became progressively slower to cook and the difference in cookability increased with increasing moisture content. At the 8% moisture level beans retained their cooking quality at 90° F. but cookability was increasingly impaired at higher moisture levels in 90° F. storage.

3. Frozen Vegetables. To supplement basic research on the histology of frozen vegetable tissue, preliminary investigations were conducted on the effects of liquid nitrogen freezing of green beans and corn. Frozen green beans with texture closely resembling that of unfrozen fresh green beans were prepared utilizing very rapid freezing that reduced breakage of cell walls and separation of cells. Lee-Kramer Shear Press measurements showed that beans frozen in liquid nitrogen were significantly firmer than those frozen in an air-blast at -20° F. Differences also existed between the air-blast frozen beans and beans frozen more slowly in a package, but the differences were very small. Organoleptic evaluations revealed that, within limits, the more rapid the freezing of green beans the firmer and more

acceptable is the frozen product. Corn that was blanched and frozen in nitrogen on the cob and then cut off was exceptionally sweet. However, after five months' storage at -10° F. an off-flavor developed.

Blanching studies with Brussels sprouts were concluded and specific recommendations were made for improving color retention in frozen sprouts, because Brussels sprouts are so large and variable in size that uniform blanching is difficult. Adequate blanching at the center of large Brussels sprouts frequently results in over-blanching the green outer leaves and this causes a loss of garden-fresh, bright green color. Preheating Brussels sprouts in water at 130° F. reduced substantially the time required for subsequent high temperature blanching. Blanching in water was found in laboratory studies to reduce blanching time requirements compared with blanching in steam at the same temperature. These findings were reported to the industry but commercial-scale operations and evaluations have not been conducted.

4. Processing Quality of Vegetables. Studies on processing characteristics of vegetable varieties are conducted cooperatively with the Washington State Agricultural Experiment Station. Evaluations are continuing on bush-type green beans which are needed to replace Blue Lake pole beans, which cannot be harvested mechanically. The bush Blue Lake varieties now available are approaching but are not equal to pole Blue Lakes grown in western Washington. The bush types are more variable in pod characteristics and breeding work will continue. Evaluations of processability will be conducted by Department scientists stationed at the Western Washington State Agricultural Experiment Station.

Experimental packs of all-white asparagus grown in central Washington indicate that good quality all-white canned asparagus can be produced there. The major commercial source of canned all-white asparagus has traditionally been in California and almost all of the product is exported to Europe. Increasing difficulties in obtaining stoop labor for California harvesting threatens to eliminate the white asparagus pack and continuation of this export trade may depend upon producing canned white asparagus in areas where harvest labor is less critical.

A new English hybrid Brussels sprout variety is being tested for commercial production in western Washington. The variety grown in this area had excellent flavor and color after processing.

5. Microbiology of Frozen Vegetables. Increasing pressure has been brought upon the frozen food industry by industry-enforced microbiological standards written into purchase agreements. Northwest frozen food packers are now willing cooperators in studies of plant sanitation and microbiology. Extensive sanitation programs exist at a number of the large freezing plants. These and the smaller plants with less adequate facilities or fewer trained personnel have cooperated informally with Department scientists to improve the general sanitary level in the industry. During the 1963 season, three

pea lines, four corn lines, three green bean lines, and one winter squash line were tested to determine the principal sources of bacterial contamination. For example, in regular cut bean lines, bacterial counts were lower than in french-cut bean lines. The difference could be traced to contamination from cutters and to the piles of sliced beans that are held back and used by check weighers to bring packages to the required weights. Such beans stand in reserve pans long enough for appreciable bacterial growth to occur. Regular cut beans, on the other hand, are filled with automatic equipment and do not require the individual check weighing of samples by workers on the processing line. Certain air cleaning equipment on pea lines was too complicated to be completely torn down for cleaning in the time usually allotted for cleanup and became a source of rather general contamination.

More basic studies on the growth of bacteria of types commonly isolated from frozen vegetables showed that reducing oxygen tension did not reduce the growth rate until the oxygen levels were less than 5%. Carbon dioxide was found to inhibit bacterial growth beyond the effect of the increase in acidity that goes with a carbon dioxide atmosphere. When systems were buffered so that no change occurred in pH, bacterial growth still was slowed by an increase in carbon dioxide concentration.

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AREA NO. 9. CASTOR, SAFFLOWER, AND OTHER WESTERN OILSEEDS--PROCESSING AND PRODUCTS

Problem. To provide valuable diversification crops for the acreage withdrawn from the production of cotton, wheat, feed grains, and other surplus crops, we must expand the markets for crops such as castor and safflower. Large amounts of safflower are exported and research is needed to insure the continuance and expansion of this promising market. Also, these crops are so new to our agricultural economy that their market potential has not been adequately developed. Castor and safflower have good potential because of the unusual properties of their oils. The possibility of large-scale increases in the production of these oilseeds would be strengthened if high-quality feed products could be developed from the oilseed meals. Basic information is needed on the composition of the oils and of the meals left after extraction of the oil, and this, in turn, requires the development of adequate analytical methodology. Rapid and accurate analytical methods are needed to control and improve the processing of the oils and meals for food, feed and industrial applications. Research on chemical conversion of the oils and evaluation of the modified products is needed to find new or improved large-volume uses. The high percentage of linoleic acid (essential fatty acid) in safflower oil points to a rapidly expanding use as a food oil. But this same fatty acid imparts a high susceptibility to autoxidation. Research is needed to stabilize safflower oil in various food products. Improved procedures for decorticating and processing castor and safflower seeds are needed. There is a particularly critical need to remove or destroy the allergenic and toxic components of castor meal which presently limit its use to fertilizer. Research to isolate and characterize the constituents in castor and safflower meals is needed to develop non-toxic, non-allergenic feed and food products of high value. Particular emphasis should be placed on developing safflower meal suitable for human consumption, opening an entry into the increasing edible protein export market. Basic and applied research is needed to prepare chemically modified products from the meals for industrial applications, to develop economical procedures for carrying out the modifications, and to evaluate the modified products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, both basic and applied research are conducted on castor and safflower seed at the Division headquarters at Albany, California and, under contract, at Tucson, Arizona, and by P.L. 480 grant funds in India. Basic, compositional studies on castor seed meal are concerned with the resolution of its water-soluble proteins and determination of the allergenic and antigenic properties of these components. Studies are conducted on the composition of castor and safflower oils and meals, and new analytical techniques are developed.

Applied research on castor meal has as its objective the development of economical methods for deallergenizing the meal without impairing its nutritive quality, to increase its value as an animal feed ingredient. Castor oil and its major constituent, ricinoleic acid, are being studied to provide for them new and improved industrial applications. Thus, methods are being developed for the preparation of various types of polyurethane foams incorporating castor oil or its derivatives. Procedures are also being devised for the preparation of chemical derivatives of ricinoleic acid, including a number of amides and phosphate esters. Several of the latter compounds may be useful for improving the flame-resistance of castor-based polyurethane foams of the type which may be used for building insulation. The utility of various polymerizable monomers from castor oil for the production of synthetic polymers for use in rubbers, plastics, etc., is being investigated under contract. Research has been initiated on the composition of new and commercially promising safflower varieties. Detailed studies are underway to evaluate variation of fatty acid, amino acid, protein, fiber, etc. with the types of seed. Oils from new and established varieties are being studied for oxidative stability which is needed for large-scale food uses. The meals are being evaluated as protein sources in animal rations. Research under contract is anticipated on the types and amounts of natural antioxidants in the various safflower seed oils.

The Federal program of research in this area totals 11.7 professional man-years, including contract research equivalent to approximately 0.7 professional man-years per year. Of this total 6.9 are assigned to chemical composition and physical properties; and 4.8 to new and improved products and processing technologies. In addition, one grant on basic studies is sponsored under P.L. 480.

PROGRAM OF STATE EXPERIMENT STATIONS

Castor and safflower are of interest due to the unusual properties of their oils and as possible replacement crops. State stations are investigating agronomic and harvesting problems. Utilization research is limited to nutritional and chemical evaluations of the castor plant being done in cooperation with USDA. Objectives include study of: the toxic and hemagglutinating protein, ricin; use of castor meal as a supplemental feed for large animals; the role of ricinine in metabolism and physiology of the plant; and isolation and identification of the compound(s) responsible for the foaming of aqueous extracts of castor beans.

There are approximately 1.8 professional man-years devoted to this study.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Detection of Allergens. A risk-free and highly specific test for human allergy, with far-reaching implications for future medical practice and

research, was reported last year as a development in the control of allergenic properties of castor seed proteins. The use of monkeys materially reduced the cost of allergy testing and, more important, eliminated hazards to human volunteers. Two hundred patients of Dr. Raphael Panzani of Marseilles, France were used in cooperative studies to indicate the extent of deallergenization of castor pomace. These studies made it possible to learn the maximum permissible level of allergen and to hyposensitize, with castor pollen extracts, patients living in the castor processing areas or on farms that used castor fertilizer. The hyposensitization allows these people to avoid suffering from allergic attacks.

Preliminary work demonstrated no cross reactions between castor allergy and chlorogenic acid allergy. The presumption of such cross reactions, as published by other workers in this area, appears to be false. Marmosets were demonstrated to be useful in the study of human castor bean allergy by the allergic serum transfer test. Since these animals can be purchased for a quarter of the price of macaques and can be kept in small animal cages in chemical laboratories or rat laboratories, this finding should make it easier for other research laboratories to enter into this type of investigation.

2. Oilseed Components. Analytical studies were initiated on 24 safflower seed samples obtained from industry, academic sources, and Crops Research Division personnel. They included the leading commercial and several new commercially promising varieties. Hull content of these safflower seeds ranged from nearly 60% for one wild variety through 40% in a typical commercial variety to 18% in experimental thin-hulled varieties. Meal and oil content of these safflowers varied inversely with the hull contents. Thus, the wild variety had only 15% meal and 30% oil while the thin-hulled experimental variety had 34% meal and 45% oil. Fatty acid analyses of the oils from these various samples are being determined.

B. New and Improved Products and Processing Technology

1. Product Developments. Polyurethane for insulating purposes in structures would be more useful if fire-retardant characteristics were built into the material. Reactive type fire retardants (that is, those containing hydroxy groups) produced foams that are self-extinguishing without lowering compressive strength seriously. Brominated castor oil was evaluated as a fire retardant polyol component. The addition of as little as 2% antimony oxide to the polyurethane system based on brominated castor oil made self-extinguishing foams. Several polyglycerol esters of castor acids were evaluated as rigid foam components. These modified castor acids may be used with a minimum addition of triisopropanolamine crosslinking agent. Foams with a wide range of open cell content were produced using the polyglycerol esters. The open cell content of such foams can thus be adjusted to meet specific industrial requirements (for example, in air filters and sponges). A re-evaluation of the insulating value of polyurethane foams was conducted

on plywood box molds which closely approximate end use conditions. Undisturbed castor-based foams had a low heat transmission which did not increase with age.

An improved mixed anhydride synthesis developed by Departmental scientists has been used to produce over 40 new substituted amides from castor-based hydroxy acids. Selected members of this series are undergoing evaluation as mold release agents and foam stabilizers. This system was also used to prepare a new class of compounds, the symmetrical anhydrides of hydroxy acids, containing mutually reactive functional groups. The anhydride from 12-hydroxystearic acid has been evaluated industrially as an epoxy resin curing agent. Fourteen acrylate esters of castor have been prepared and are being purified and characterized before polymerization studies are undertaken in contract research at the University of Arizona. Work there on the co- and homo-polymerization of vinyl esters of castor derivatives is essentially completed, and evaluation of the polymers is planned. The mechanism of the catalytic isomerization of methyl ricinoleate to methyl 12-ketostearate has been elucidated. Information gained has aided in the design of more economic routes to this potentially valuable industrial compound.

2. Animal Feeds. Castor pomace samples were deallergenized by moderate treatment with ammonia and water and found to be non-allergenic by monkey tests which were confirmed with 200 human patients in Marseilles, France in collaborative work with Dr. Panzani. The mechanical difficulties of scaling up to laboratory deallergenization of pomace with ammonia have been overcome and complete deallergenization as judged by pharmacological criteria was accomplished using sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, or gaseous ammonia. With gaseous ammonia it was necessary to form a water slurry of the pomace before treatment Other reagents were used in solution so that slurries were formed during the process. In-plant control methods for deallergenization will need simplification. Steam treatments of castor pomace in the absence of the other reagents did not completely remove the allergenicity.

Sufficient castor pomace was deallergenized using ammonia gas in a high water-to-solids ratio to permit a chick bioassay of the feed value. No unusual symptoms attributable to the ammoniation appeared in the birds and the protein appeared to be acceptable for feed. Plant-scale trials of deallergenization by cooperation with a commercial producer of castor oil are planned for the near future. Attempts are being made to find pressures and temperatures more suitable for commercial deallergenization.

Safflower meal supplemented with adequate lysine yields extremely high growth rates in chicks. Feed efficiency is impaired, presumably by residual hull fiber in the partially decorticated meal, and additional studies on low-fiber meal are anticipated.

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AREA NO. 10. SUGAR BEETS-- PROCESSING AND PRODUCTS

Problem. Sugar beets are mainly processed for sugar; a very small proportion is used for livestock feed. Sugar beets are declining in sugar content and rising in impurities. The traditional processing methods for sugar manufacture cannot cope efficiently with beets whose lower quality is due in part to excess nitrogen fertilizer, used to improve tonnage yields. Improved processing procedures should benefit both the growers and processors. It is known that small concentrations of certain chemicals in beets affect processing quality but not enough information is yet available to devise new economical procedures for high-impurity beets. Because costs of producing beets and processing sugar are rising whereas per capita consumption and price of sugar are essentially constant, all factors important to utilizing the crop must be examined to improve processing. There is still much to be learned about the composition of sugar beets, juices, pulp, and crude sugar. Sugar losses resulting from spoilage and respiration of beets held at processing plants cannot be prevented by existing methods. Only an expanded research program can provide the needed information at an early date.

USDA AND COOPERATIVE PROGRAM

Both basic research and process development studies on sugar beets are being conducted in the Western Utilization Research and Development Division's headquarters laboratory at Albany, California and under P.L. 480 grants in Calcutta, India and Jerusalem, Israel. The basic research program involves a comprehensive study of the naturally-occurring sugar beet and beet juice constituents, both carbohydrate and non-carbohydrate. Biochemical studies of the carbohydrate constituents aim to determine their origin leading to the development of methods for the reduction or elimination of those which decrease sucrose. Chemical studies of the non-carbohydrate constituents provide information to ameliorate effects of adverse constituents. Although Federal research on new products from sucrose (sucrochemicals) has been terminated, studies continue under P.L. 480 grants on the reactions of sucrose with vegetable and animal fats and oils, to produce new and useful compounds having special hydrophilic and lipophilic properties. Processing research on sugar beets deals with the effects of the many variables which influence the efficiency of recovery of sucrose. Pilot-scale sugar beet processing facilities are used to test these processing variables and to evaluate new and improved processing techniques.

The Federal program of research in this area totals 7.8 professional man-years. Of this total, 3.6 are assigned to chemical composition and physical properties; 4.2 to new and improved products and processing technology. In addition, the Division sponsors, under P.L. 480, one basic research project and one on product developments.

PROGRAM OF STATE EXPERIMENT STATIONS

The station program on sugar beet utilization is concerned with chemical composition of the beets. The effects of management, genetics, and environmental factors on yield and quality of sugar beets, including sucrose content of root and purity of juice are being determined. Other research, while serving projects designed to consider possibilities of growing beets in several new areas, also provides data on composition and the influence of environment and practices of fertilization and management on yield and quality of sugar beets.

The total research effort devoted to this work is about 5.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Sugar Beet Composition. Carbohydrate transformations during storage of sugar beets between harvest and processing are important to the sugar processor. Indirect losses of sugars and production of new compounds deleterious to the recovery of sugar have not been completely evaluated. It is known that these new compounds make it harder to extract sucrose. Investigations of the effect of processing methods on the accumulation of non-sucrose carbohydrates depend upon the development of better analytical methods. Thin-layer chromatography promises to be a good method to observe carbohydrates from beets and stored beet juices and the changes that occur in them. Galactinol has not been found in stored beet juices from California but kestose and raffinose accumulate after less than two months' cold storage of beets. When sugar beets were stored in the cold for nearly three months to attain high levels of raffinose and kestose and then returned to room temperature, kestose and raffinose appeared to diminish. Under the room temperature storage conditions reducing sugar rose to an unacceptable high value.

An analytical study of constituents of California sugar beets was concluded. Sucrose, reducing sugar, betaine, anions, sodium, potassium, chloride, marc, and soluble nitrogen were determined. Simple correlations of the non-sugar constituents against purity measurements of beet juice were highly significant and suggest the need for new methods of determining ionic impurities. The highly negative correlation of sodium and potassium with juice purity offers a possibility for routine measurement of these ions as an index of juice quality.

Preparation of trimethylsilyl ether derivatives of molasses carbohydrates allows a satisfactory qualitative and semi-quantitative analysis by use of gas-liquid chromatography. Attempts will be made to further develop this technique into a quantitative method for beet carbohydrates.

B. New and Improved Products and Processing Technology

1. Juice Diffusion. Further studies on lime treatment of sugar beet cossettes before diffusion showed that the juice produced is very much easier to clarify than juice from untreated cossettes. The overall lime use was reduced in laboratory tests from 2 or more to 1% of the weight of beets without sacrifice of clarity, filtration rate, sedimentation rate, or purity. Acetate ion increased, however, which means that sodium carbonate addition would be necessary in order to reduce lime salts. The pulp produced from this lime process was hard and tough and could be pressed to higher solids content with a hydraulic press than non-treated pulp. Factory-scale pressing tests with the treated pulp were drier, too, but clogged the presses some. Some of the older presses used in processing plants may be inadequate to take advantage of this improved procedure. Tests in commercial pressing equipment having a positive feed would be desirable and would demonstrate to the industry the savings in drying costs that could be made from pressing a tougher pulp.

2. Beet Juice Purification. Experiments using ion exchange and ion exclusion were conducted and appear promising as means for separating non-sugars from sucrose in beet sugar manufacturing. Small molecules such as amino acids diffuse into the ion exclusion resin and are partially eliminated whereas large molecules such as colored compounds and colloids from sugar beet juice pass through the resin and are eliminated. The resin therefore rejects non-sugar molecules in two ways which promise higher elimination efficiency than other purification schemes. In static resin columns this separation is accompanied by large dilution of the sugar. A practical means is being sought to develop a continuous countercurrent movement of juice and resin.

Sugar beet pulp from limed cossettes or pulp treated with lime under controlled conditions was converted to a thickening agent with possibilities for use in fire fighting compositions containing ammonium phosphate. Studies are continuing with tests conducted in cooperation with the United States Forest Service and the California State Division of Forestry. Variability of available pulp creates some uncertainty as to the best method for preparation of the thickening agent, and investigations will continue towards developing a general process for the pulp.

3. Sucrochemicals. The domestic Federal program of research on sucrochemicals was terminated in response to recommendations of the Sugar Research and Marketing Advisory Committee in 1963. A similar stand was taken by the Oilseed, Peanut and Sugar Crops Research Advisory Committee in 1964. Prior to termination of research on sucrochemicals, negotiations had taken place to provide P.L. 480 grants for work in this area at the Tropical Products Institute in London, England to develop surfactant compounds using sugar and animal and vegetable fats and at Jadavpur University in Calcutta, India to develop modified sugars that could be used for the synthesis of useful plastic substances. In the English work combinations of sucrose and low-cost

simple fatty acids were made but surfactant qualities were not high. Surfactant compounds using more complex hydroxy-substituted fatty acids such as ricinoleic acid from castor oil were prepared. High-quality surfactants prepared with these complex compounds had the high biodegradability of the simpler compounds. This project has been concluded.

The basic research in India has continued on the reaction of sucrose with sulfonochloride and similar compounds. Starting with tri-O-tosyl sucrose, new compounds with reactive end groups were synthesized including hydrazine and azido sucrose. Reactive end groups such as these form the basis for linking monomers together to make plastics.

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AREA NO. 11. POULTRY-- PROCESSING AND PRODUCTS

Problem. The $1.6 billion poultry meat industry operates on very narrow profit margins. This industry is confronted with the problem of converting continually increasing amounts of poultry into a wide variety of products having high quality and improved convenience, at costs attractive to consumers and remunerative to the poultry grower. More information on the properties and processing of poultry is needed to enable us to better utilize poultry in a variety of forms attractive to consumers. Increased utilization of poultry would also serve toward eliminating our feed grain surplus, increasing returns to farmers and providing better products for American consumers.

Although poultry is an efficient converter of feed to meat, more grain is used by poultry per calorie of food produced than by any other commercial animal because a high percentage of the poultry diet is grain and because poultry meat contains exceedingly little fat. Furthermore, one-fourth of all grain fed to animals is used for poultry and egg production. Hence, increased consumption of poultry products would be an effective means of increasing markets for surplus grain. Also, the efficiency of feed utilization by poultry makes possible low prices within reach of more consumers. A still further benefit would arise from the increased use of poultry by improving the nutrition of consumers having diets now low in animal protein.

The consumption of poultry has steadily increased from a 1947-1949 average of 22 lbs. per capita to 39 lbs. for 1963. This important increase has involved price, quality of product, availability, and disposable income. Because of the current low profit margin it is impractical to increase consumption by lowering farm prices. Increased demand for and consumption of poultry will require higher quality and more convenient products and a greater variety to meet the desires of the modern consumer. However, in addition to greater returns from increased demand, a greater profit margin for the farmer can, of course, come from greater efficiencies in processing.

The trend toward convenience foods and further processing has primarily led to precooked poultry products which are generally less stable, more subject to warmed-over flavors, and more likely to provide texture problems than uncooked items. With the expansion of operation and the emphasis on continuous, more efficient processing, need has arisen for improved processing procedures for feather removal, chilling, tenderization, freezing, deboning, and commercial cooking. Lowering the cost and improving the quality of products that can be stored at ambient temperatures, such as canned, dried, cured, and irradiated products, offer potential for poultry utilization in domestic and export markets. As a foundation for applied studies, further knowledge is needed on the chemical nature of flavor and flavor changes in

processing and storage, on tenderness development, and on proteins, lipids, and other components.

USDA AND COOPERATIVE PROGRAM

Basic and applied research on poultry meat and poultry meat products are conducted at the Division headquarters at Albany, California and by contract in East Lansing, Michigan, Madison, Wisconsin, and Berkeley, California. Fundamental studies on poultry flavor are concerned with the identification of flavor precursor constituents in poultry meat and in the isolation and identification of volatile flavor components developed during the cooking of poultry. The chemistry of muscle protein and post-mortem chemical changes are investigated relative to the tenderness and other quality characteristics of poultry. The basic physiological character of feather release mechanism in fowls is studied to provide a foundation for improved feather removal. Applied research is conducted on the stability of cold-tolerant organisms; special problems of flavor, texture and stability of precooked frozen foods; and processing factors that influence tenderness of poultry meat.

The Federal program of research in this area totals 14.9 professional man-years, including contract research equivalent to approximately 1.8 professional man-years per year. Of this number, 8.3 are assigned to chemical composition and physical properties; 6.6 to new and improved food products and processing technology.

PROGRAM OF STATE EXPERIMENT STATIONS

Both basic and applied researches on poultry and turkey meat and meat products are being conducted by State stations. A problem of major concern is preserving or improving the initial quality. This work is directed to maintaining quality during off-farm handling, killing, dressing, eviscerating, cutting-up, chilling and packing fresh poultry. The effect of the various operations on the product is measured and related to development of principles or design features which will best maintain quality. Factors such as wholesomeness, microflora, dressing percentages, condemnation losses, disease effects, physiological and biological changes during post-mortem aging, and tenderness are evaluated.

Efficient processing and utilization are dependent upon development of more efficient work methods, equipment and facilities for handling, processing and packaging poultry and poultry products. Pressures for high speed, continuous processing, i.e., continuous chilling, give added impetus to this research. Methods of feather release, removal and scalding are being studied. Radioisotopes are employed in investigations of blood removal. Equipment is designed, developed and tested. Effects on fat, on bone darkening, weight losses and nutritive value are followed.

A number of fundamental and applied studies are directed to elucidating the chemical and physical properties and composition of poultry and turkey meat.

Effects of diet, processing treatment, handling and storage on the various chemical components are measured and compared by following carcass lipids, protein and mineral content and distribution. Special attention is being given the biological value of the protein and other constituents. Other research deals with the specific ionic shifts associated with post-mortem changes in poultry meat.

Flavor and nutritional value are important attributes of poultry products. Investigations of the chemical composition of poultry products are being carried out to determine the chemical components of poultry products which are related to or produce flavor or off-flavors in poultry meat. Because of the adverse effects of off-flavors on consumer satisfaction, attempts are being made to reproduce conditions responsible for off-flavors and/or odors in market poultry in order to confirm the relationship to practices in processing or distribution and to devise means of control.

Basic microbiological studies are being directed to establishing the source of organisms, and the natural flora present on poultry; to the source and effects of organisms found in bruised or diseased tissue; to the build-up of organisms in the scald tank water and along the processing line; and to means for extending shelf-life by retarding bacterial growth in packaged poultry and in poultry products. Another phase of the work deals with growth, survival and control of potentially pathogenic organisms.

New products research is directed to accumulating fundamental information basic to development of new or improved poultry and turkey products. Studies include determining yield; methods for meat recovery; and methods for curing, smoking, cooking, and shelf-life extension of poultry products. New approaches are sought to improve form, texture, flavor, juiciness, and quality of products. Precooked, freeze-dried turkey and poultry products are being investigated.

Economic feasibility and market tests are being conducted to determine possibilities of commercial productions of new or improved products designed to increase the use of poultry. Economic efficiency of turkey and poultry processing operations are also evaluated.

A portion of the research on poultry products is conducted under the regional project NCM-7.

The total research effort in poultry and turkey products, processing and utilization is approximately 17.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Post-Mortem Biochemistry and Tenderness. Basic investigations are continuing on the proteins of poultry muscle and their reactions that affect the tenderness and other texture qualities of poultry meat. It was previously

shown that rate of muscle glycolysis correlated with and was possibly involved in tenderness achieved by aging slaughtered poultry. In the absence of glycogen, the meat was found to be more tender. Some causes of difference in glycogen level were found. The level was highest in birds that had been anesthetized before slaughter and significantly lower in birds immobilized by electric stunning during bleeding or allowed to struggle immediately before slaughter. The concentration of creatine phosphate, an important reactant in the glycolysis of post-mortem muscle, was found to parallel the glycogen level. Electrically stunned chickens had almost 90% less creatine phosphate than those that had been anesthetized before slaughter. Thus slaughter conditions may affect toughness.

The large variation in tenderness from one bird to another shortly after slaughter remains as a challenge to research. Rates of pH decline in chicken breast muscle immediately after slaughter also showed a considerable variation from one bird to another. However, when pH rate of decline and tenderness as measured by shear press were evaluated, the two showed no strong correlation. Processing efficiency could be materially increased if aging time could be reduced and this would be possible if handling and processing could be adjusted so that all birds had tenderness equivalent to the most tender ones. No empirical method for reducing aging time has been found and these basic investigations will continue to search for useful leads.

Correlations have been established between objective shear force measurements and subjective response of average panel members over the range of toughness in turkey meat that can result from inadequate aging. This now permits establishment of minimum aging times for a given required percentage of tender birds within a group, and also defines average acceptability in terms of an objective test.

2. Chemistry of Poultry Flavor. Basic research is continuing on the isolation and identification of volatile constituents of poultry and the relationship of individual compounds and groups of compounds to flavor. More than 200 compounds in the steam-volatile, isopentane soluble fraction of boiled chicken meat were indicated by fractionation with a sensitive gas chromatograph. By coupling the gas chromatograph to a mass spectrograph, about 30 compounds, comprising aldehydes, ketones, and hitherto unrecognized aliphatic and aromatic hydrocarbons, have been tentatively identified. Investigations also revealed that hydrogen sulfide evolves from the amino acids, cystine and cysteine, of muscle protein during cooking. Only about 1% of the amino acids was transformed per hour, hence the evolution of hydrogen sulfide will continue at nearly a constant rate for long periods of cooking. The rate of evolution was increased by increasing muscle pH. Hydrogen sulfide levels in chicken broth and meat were determined at various temperatures. Taste tests were conducted with samples at various natural hydrogen sulfide levels and samples to which hydrogen sulfide was added. Significant correlations between hydrogen sulfide level and chicken flavor have not yet been found but the investigation is continuing. Such an odorous substance might contribute to the flavor of cooked chicken even though much of it escapes during normal cooking.

3. Pharmacological Investigations. The poultry industry produces approximately 1 billion pounds of chicken backs and necks annually. Because of low market value of this material, it has been proposed that homogenized chicken backs and necks be incorporated in gravy, soup mixes, and new poultry products. Feeding tests with weanling rats were conducted to determine the availability of fluorine from ground bone meal prepared from dried chicken bones. Chicken bone meal was added to provide a range of dietary levels. For comparative purposes, identical levels of fluorine were provided in control weanling rat diets using sodium fluoride rather than the ground chicken bones. At the end of 190 days the results as measured by bleaching of rat incisor teeth were identical for the two sources of fluorine. These data support the contention that the fluoride in bone meal is as readily available as fluoride from sodium fluoride salt in the diet. The use of chicken back and neck bones for human food products must be evaluated relative to the total fluorine ingestion that is potential for any area or set of circumstances.

4. Physiology of Feather Release. The physiological mechanisms that control feather tightening and release are being investigated in contract research at Michigan State University in East Lansing. Further experimental evidence was found of muscle-controlled tension in feather follicles and its importance in establishing the force required to pull feathers. Anesthetization caused a 25-35% decrease in intrafollicular pressure which paralleled a decrease in feather pulling force. Mechanical or electrical stimulation of the nervous system increased intrafollicular pressures. Death of anesthetized birds by neck breaking led to a rise in intrafollicular pressure and feather pulling force.

Both sympathetic and parasympathetic nervous systems play a role in control of feather tightness. Sympatholytic and parasympatholytic drugs (agents which block or inhibit the respective nervous systems) produced decreases in intrafollicular pressure and in feather pulling force. Sympathomimetic and parasympathomimetic drugs (agents which imitate or stimulate the normal actions of the two respective nervous systems) were able to increase the intrafollicular pressure and feather pulling force of chickens in which the pressure and force had been reduced experimentally by anesthetization.

B. New and Improved Food Products and Processing Technology

1. Freeze-drying of Poultry. Two new vacuum dryer designs have been evaluated in preliminary studies. A fin tube dryer, in which heat exchange fins are located in the body of a rotating dryer so that the product tumbles against them during the drying operation, reduced drying time. New surfaces are continuously exposed to receive heat from the sources in the vacuum chamber. The dryer was installed, tested, and found to operate satisfactorily. An experimental model of a product-in-tube dryer was also built and proved satisfactory in preliminary trials. Use of input and outlet reservoirs gave a semi-continuous operation that was long enough to simulate continuous drying. In this dryer the product tumbles in tubes surrounded by the heat source (hot air, steam, or hot water).

These two new dryers plus the conventional vacuum shelf dryer and the tumbling dryer, constructed sometime ago, provide a wide range of heat transfer possibilities. Engineering data on freeze drying were obtained and will soon be available on a broad range not feasible before.

Commercial vacuum-dried poultry samples were evaluated. The products were inferior to fresh or frozen poultry meat in their dryness, toughness, and lack of cohesiveness. The defects were more noticeable in dark meat than in light meat. The procedures for organoleptic evaluation of these products were established and will be used in experimentally produced vacuum-dried poultry meat in search of ways to process chicken better.

A measurement of the expression of juice from poultry muscle tissue under standard conditions (Grau-Hamm press method) was applied to the measurement of juiciness and texture in raw, frozen thawed, cooked, and freeze-dried rehydrated poultry meat. Only a small sample (300 mg.) is necessary and the method lends itself to study of effects of processes on quality of freeze-dried poultry. The press characteristics of four commercial freeze-dried cooked poultry meat samples were quite similar.

2. Low-temperature Microbiology. Polyphosphates, used in the cured meat industry to improve texture and moistness, retard spoilage of raw poultry at refrigeration temperatures. Basic studies of the mechanism of the microbial inhibition showed that the polyphosphates inhibit by binding (chelating), and thus making unavailable, various trace elements required for growth. The inhibition was readily reversed by adding metal salts or by adding competing chelating agents that are small enough to enter the cell and be metabolized. Both the pyoverdines, which are chelators produced by certain psychrophiles, and peptone were effective. In small scale tests, treatment of raw chicken with polyphosphate solutions in a variety of ways increased the keeping time at 36° F. 20 to 70%. The cost of treatments thus far devised would be an important consideration where shelf life is the only benefit to be derived from the treatments.

An improved design was developed for our temperature gradient incubator that is used for the efficient determination of temperature limits of growth and survival of microorganisms.

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AREA NO. 12. EGGS-- PROCESSING AND PRODUCTS

Problem. The nearly $2 billion egg industry is periodically faced with burdensome surpluses that drive prices below the break-even point for many producers. The industry is also faced with declining per capita consumption. The estimate for 1963 is down 18% from the 1947-1949 average consumption. Because the demand for table eggs is inelastic, the increased utilization of eggs must come in the form of new egg products that compete by means of quality and convenience. Adequate knowledge is lacking of the properties, processing characteristics, and new product potentials of eggs to develop new markets. Present outlets for the 10% of egg production that is frozen or dried include the baking, confectionery, salad dressing, noodle, and baby food trades. Modified and new products emphasizing quality and convenience are needed to increase acceptance of egg products by these industries in order to compete successfully with egg substitutes.

Increased utilization of eggs would not only benefit the producer, but would also diminish our feed grain surpluses since poultry and egg production account for about one-fourth of all grain fed to animals. Improved egg-containing products would benefit the producer in three ways: by providing an increasingly useful buffer for stabilizing egg prices; by providing additional uses and outlets for eggs; and by providing more remunerative outlets for wholesome eggs that are unsuitable for table use because of appearance or handling characteristics.

Egg processors have four general problems. First, the potential of yolk-containing solids in convenience foods can be fulfilled only with improvement of flavor stability, of dispersibility, and freedom from pathogenic Salmonella bacteria. Secondly, the processing costs of whites should be reduced and their utility improved. Third, further basic research on egg composition and components is essential to reach an understanding of physical and chemical changes induced by processing and storage and thus provide a rational basis for devising improved processes and products. Fourth, formulation studies designed to incorporate eggs into new household and institutional convenience products, are needed. This last study must encompass a full appraisal of physical, chemical, and microbiological problems peculiar to the formulated products.

USDA AND COOPERATIVE PROGRAM

In the Western Utilization Research and Development Division, a broad program of basic and applied research is conducted at the Division headquarters at Albany, California; by contract in Ames, Iowa, Ithaca New York, and Davis, California; and by grant funds under P.L. 480 in France, Australia, and India. Fundamental research is conducted on egg proteins and their relations to the functional properties and quality of eggs, on egg lipids and their

role in off-flavor development in yolk solids, on the mechanism of bacterial penetration and survival in eggs, and on the bactericidal, antiseptic, anti-inflammatory, and food preservative properties of lysozymes and other components from eggs. Applied research is conducted on the stabilization of yolk-containing solids to increase the usefulness of eggs in dry mixes and other convenience foods, on new and improved drying procedures to make dried egg fractions and products more readily and more completely dispersible, on various methods of controlling Salmonella in eggs, and on factors in the handling of shell eggs that affect egg product quality and cost.

The Federal program of research in this area totals 16.1 professional man-years, including contract research equivalent to approximately 2.1 professional man-years per year. Of this number, 2.3 are assigned to chemical composition and physical properties, and 13.8 to new and improved food products and processing technology. In addition, three research grants on basic problems are supported by P.L. 480 funds.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations maintain a continuing program of basic and applied research on egg and egg product utilization. It begins with the concern for quality of the freshly laid egg and extends through to work designed to maintain the storage life of novel egg products.

Research on initial quality includes: evaluation of the influence of breed and strain of laying hen on the chemical properties of eggs and the relationship of these constituents to the functional properties of the egg; study of the effects of diet, such as the cause and prevention of egg yolk discoloration resulting from feeding cottonseed oil to laying hens; and determining the effect of various washing and shell treating techniques on the quality, nutritive value, stability and public health aspects of shell eggs and egg products. Other research is being conducted to determine the influence that shell characteristics, temperature, humidity, holding time in storage, and packaging have upon egg quality and use. The relationship of shell thickness and breaking strength to retention of initial quality and freedom from bacterial contamination is receiving special attention.

Basic investigations designed to more fully characterize egg composition and elucidate the relationships between chemical composition, structure, biological activity and functional properties are in progress. Studies are being made of the chalaziferous layer and of the vitelline membrane to determine roles each play in the migration of materials into and out of the yolk. A study of the comparative biochemistry of the proteins of eggs is going forward. Study of the protein fractions of eggs may lead to increased utilization of eggs by providing a better understanding of the factors affecting foaming, coagulative and emulsifying properties of eggs and egg products. Investigations of the cholesterol and other constituents and of factors affecting these constituents in eggs are providing basic data which are useful in considering health-related questions.

Microbiological investigations include: study of bacterial spoilage of shell eggs; determination of the mode of action of proteolytic and lipolytic enzymes of psychrophiles in the deterioration of eggs and the influence of shell membranes in resisting bacterial penetration; and investigation of means for control of Salmonella in egg products. Other work relates to methods for controlling bacterial contamination in new products.

Among the efforts to develop new egg products is work designed to develop shell-less eggs. Considerable attention is also being given frozen eggs and frozen egg products.

Designing, constructing and testing an experimental egg cleaning machine is a phase of the processing techniques research. Other processing research seeks to evaluate egg washing methods. Another study, which is searching for means to improve the quality of eggs from small plants, is engaged with developing and evaluating procedures for pasteurizing liquid eggs. Use of ultrasonic techniques for egg cleaning and processes for freezing eggs are also being studied.

Finally, the costs, efficiency and economic feasibility of egg processing operations are being studied. These engineering-economic studies consider alternative methods of utilization of eggs and the advantages of each.

A part of the work on egg utilization is conducted under the regional project NCM-7.

There are about 10.9 professional man-years devoted to egg utilization researches at State Agricultural Experiment Stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Oxidative Changes in Yolk Lipids. Contract research on the oxidative mechanisms involved in egg lipid systems was completed at the Hormel Institute in Austin, Minnesota. At present only organoleptic tests reliably detect oxidative flavor deterioration in yolk-containing egg powders. It was found that the increase during storage in amount of volatiles absorbing at 280 millimicron has potential as an objective index of flavor deterioration. Other objective measurements showed poorer correlation with the organoleptic results. The polyunsaturated fatty acids and neutral lipid fractions from stored eggs showed essentially no deterioration, while the intact phospholipid system showed measurable deterioration. Isolated egg phospholipid systems were found to autoxidize much more rapidly than methyl esters of highly unsaturated fatty acids. Hydrogenated egg lecithin promoted oxidation in a model system. These results emphasize the importance of the phospholipids in research on oxidative deterioration of eggs and other commodities also.

2. Egg Proteins. Basic investigations on lysozymes from various sources including eggs are continuing at the University of Paris, France, supported by P.L. 480 funds. Lysozymes are enzymes with bactericidal and other biological properties. Lysozymes were separated by ion exchange chromatography from turkey and goose egg white and other animal sources to compare them with lysozymes from hen eggs in amino acid composition and other characteristics. Relationships between chemical structure and biological activity of egg white lysozyme were investigated.

Research was initiated at the Indian Institute of Science in Bangalore supported by P.L. 480 funds to determine changes in properties caused by freezing of hen egg yolk proteins. A determination is being made of the specific changes in proteins and lipoproteins resulting from freezing and thawing hen egg yolk.

B. New and Improved Products and Processing Technology

1. Bacterial Spoilage of Eggs and Egg Products. Over 10% of eggs produced are utilized in frozen and dried products. Continuation of egg processing and its expansion in both domestic and export markets is essential to the economic soundness of the egg industry, but pathogenic Salmonella threatens the outlet and limits the expansion. Pasteurization to control Salmonella is under investigation. In these studies, Salmonella strains of average heat resistance at inoculations of 10^6 to 10^7 per ml. in whole egg were easily destroyed in conventional plate and holding tube equipment by treating at 140° F. for two minutes or by flash heating to 160° F. for approximately three seconds. Such conditions did not materially reduce the usefulness of eggs. Studies with a heat resistant strain, (S. senftenberg 775W), indicate that only higher temperatures or longer treatments would kill contamination that might occur. Three and one-half minutes at 150° F., which may be a practical limit of pasteurization for whole eggs, did not consistently and completely destroy a 10^4 per ml. inoculation of this strain. Steam injection heating for about three seconds at 165° F. did not harm the functional properties of eggs. Commercial-type pasteurization (142 to 143° F. for 3-1/2 min.) did not harm fresh whole egg even for the demanding requirements of sponge cakes. On the other hand, when frozen whole egg was pasteurized whip time had to be increased by a third to make an acceptable sponge cake. Its performance in other cakes, for example commercial-type sponge cakes, which are less dependent upon egg quality than true sponge cake, and layer cakes, was unaffected. Combinations of homogenization and careful control of heat pasteurization condition can be used to correct damage to the foaming power of pasteurized frozen whole egg liquid, but yield a much thinner product on thawing.

Pasteurization of unmodified liquid egg whites in commercial plate heater and holding tube equipment above 134° F. for two minutes caused an increase in turbidity and viscosity of whites. The most severe treatment that did not visibly change egg whites was 133° F. for two minutes, but this treatment damaged foaming power in angel cake. The loss in foaming power for angel

cake could be corrected by additives. The cake volume was unaffected. Treatment at 133° F. for two minutes is marginal and the absence of Salmonella from the final product would have to be verified bacteriologically.

Basic studies of the heat stability of individual egg white proteins were conducted in an attempt to find conditions that would permit egg white pasteurization by the whole egg procedure (140° to 144° F. for 3-1/2 to 4 min.). All of the proteins but one (conalbumin) were found to be adequately stable at pH 7 and this one could be stabilized sufficiently to withstand these conditions by addition of an iron salt or certain other metal salts. Aluminum salts were especially suitable because they are not toxic and they cause no color change as do the iron salts. Applied tests were conducted both in the pilot plant and in commercial plants. Excellent kills of salmonellae were achieved at 140° F. (as reported already for whole egg above) without coagulation or increase in viscosity of the white. Whip times were increased but could be corrected by adding certain conventional whipping aids. Angel cake volumes and textures were essentially unaltered.

Contract research on Salmonella contamination in eggs was initiated at Iowa State University at Ames. A technique for detection of Salmonella by fluorescent antibodies promises to give results in 24 hours comparable to the three- to four-day enrichment differentiation and confirmation tests now in use. Relationship of Salmonella infection in laying hens to egg contamination and relative contribution of egg infections and plant equipment sanitary levels to the presence of Salmonella in egg products are being studied.

2. Egg Powders. Addition of sucrose to whole egg and yolk before spray drying has long been known to yield powders with improved performance value and stability. However, at levels commonly employed, sucrose addition greatly accelerates and intensifies off-flavor development in air-packed powders even at refrigerator temperatures. Recently, in commercial practice, low dextrose equivalent corn syrup solids have been substituted for sucrose at the same level to improve flavor stability. This has led to the general belief that sucrose addition induces instability over a wide range of levels and, conversely, that low dextrose equivalent corn syrup solids do not. Present findings discount these beliefs and show that under mild oxidative conditions, comparable flavor stabilities and instabilities can be achieved with both sucrose and various corn syrup solids but at different levels of added carbohydrate. Typically, in each case, flavor stability was gradually improved at lower levels of added carbohydrates reaching a maximum flavor stability followed by an abrupt transition to marked flavor instability at succeeding levels. This transition corresponded to a change in the physical state of the egg lipids from one of coalescence where the lipids were readily extractable with mild solvent to a finely dispersed or emulsified state where the lipids were virtually nonextractable. Chemical indices of oxidative flavor deterioration (peroxide, carbonyl, TBA) correlated fairly well with organoleptic findings for powders stored at low temperatures.

Flavor and chemical stability relationships were determined for yolk, fortified whole egg, and whole egg solids as a function of graded levels of added sucrose, and 24 and 42 dextrose equivalent corn syrup solids. Use of corn syrup solids gave equally good protection as sucrose against browning reactions at elevated temperatures despite their containing substantial quantities of glucose and other reducing sugars. It is now feasible to select levels of any of the carbohydrates studied which yield dried products that combine good retention and stability of performance quality with outstanding flavor stability.

3. Precooked Frozen Foods. Studies of the effects of ingredients on stability of foam structure after freezing and frozen storage are continuing since successful freezing of such products would increase the utilization of eggs in this rapidly expanding industry. The effect of individual constituents on volume retention of products having a structure dependent on a stable egg foam is being evaluated. Foam stability was increased by increasing the solids content of whole egg souffles for freezing. Storage temperature was also important. At -30° F. products shrank, particularly those low in solids. Volume changed little at storage temperature of 10° F. but this temperature was unsuitable for souffles because they develop off-flavors within six months. Storage at 0° F. was, therefore, selected as optimum from both flavor and texture standpoint. Variations in formulas and baking conditions were studied for a dessert souffle made of egg white and sugar, to develop a product that was reasonably stable to freezing and storage. Addition of a sauce thickened with flour, similar to the type used successfully in a whole egg souffle, was not satisfactory in combination with an egg white foam.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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New and Improved Products and Processing Technology

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AREA NO. 13. PHARMACOLOGY

Problem. Advances in agricultural science and processing technology necessitate the use of an increasing number of new chemical compounds whose safety must be established. The mutual interests of both the producers and the consumers of agricultural products, demand that public agencies participate in securing unequivocal evidence of safety before products of advanced technology are marketed. This responsibility is particularly acute where a public agency, such as the Department of Agriculture, contributes to technological developments that result in intentional or unintentional addition of untested components into foods, feeds, or into materials contacting the persons of consumers, or developments that result in the introduction, concentration, or modification of natural components in a way that may have an adverse physiological effect on consumers. Types of materials that require continuing surveillance include food additives, inadvertent residues of pesticides and other useful agricultural chemicals, antibiotics and medicinals, and the naturally occurring chemical constituents of physiological importance. In the areas of interest to Agricultural Utilization Research and, in particular, in connection with process and product developments of the four Utilization Research Divisions, such compounds must be tested by short- and long-term ingestion in experimental animals, such as rats and dogs, to secure toxicological data required by the Federal Food and Drug Administration to establish safety and legal certification for their use. The unequivocal establishment of safety for any useful chemical involves much more than merely conducting animal feeding tests on a routine service basis. It often requires original chemical analytical procedures and metabolic fate studies in experimental animals, as well as new methodology and observational techniques, to study of new chemicals. Each assignment in this field is a new area for original, often fundamental research.

A new dimension has recently been added to the problems in pharmacology facing the Department of Agriculture by the recent discovery that the mold Aspergillus flavus growing on peanut meal produces toxic compounds which are carcinogenic. Since it is unlikely that production of toxic compounds is limited to Aspergillus flavus, the compounds produced by any mold on any agricultural product should be tested for acute and chronic toxicities, metabolic fate, and mechanism of producing physiological effects.

USDA AND COOPERATIVE PROGRAM

Pharmacological investigations supporting the Department's utilization research and development program are conducted in the Western Utilization Research and Development Division at Albany, California. Agricultural products, and additives required to preserve or otherwise treat them, are investigated as they may cause toxic or allergenic reactions. Laboratory methods for discovering the metabolic fate of chemical compounds in animal physiology are developed and applied to problems in the utilization of farm products.

Plant constituents that exert deleterious or beneficial effects on animal growth are studied to determine quantitative responses.

The Federal program of research in this area totals 10.0 professional man-years assigned to pharmacology investigations.

PROGRAM OF STATE EXPERIMENT STATIONS

As public agencies, the State agricultural experiment stations are concerned with the safety of various agricultural commodities. As a result of this concern, a continuing program of research on the pharmacological aspects of various commodities is conducted.

Research is in progress on the biological and chemical properties of toxic substances which are produced by growth of molds in animal feeds. Much effort is being devoted to concentrating, isolating, and identifying substances which are produced on moldy protein concentrates and to determining their mode of action in retarding growth. Other research is focused upon identification and characterization of the fungi associated with outbreaks of toxicities and upon development of techniques for detection of fungal products in feeds and foods.

A second phase of the pharmacological research program deals with the biochemistry of certain plant constituents which are toxic. Isolation, characterization, and identification of the constituents having toxic effects is the first step. Work is devoted to developing bioassay techniques and to determining toxic dosages and levels. Mechanisms of biosynthesis and detoxification are also studied. Certain purified fractions, for example, proteins or other toxic compounds, are obtained or synthesized and tested. Specific examples are the studies dealing with coumarin in sweet clover; isolation and identification of the antipyridoxine factor in flaxseed; and of symptoms of toxicity in animals due to ingestion of fescue grass in a cooperative study with the Northern Utilization laboratory.

In still other researches, chemical composition of Delphinium bicolor is being investigated with special emphasis on the toxicity of the alkaloids to animals. Variation in the alkaloid content of selected species of lupines with stage of maturity, soil type and environment is being evaluated. The seeds of various native western plants of agricultural importance are being studied to determine the presence or absence of specific hemagglutinating substances. Other researches involve isolation of the organic selenium compounds in Stanleya bipinnata and determination of their biological activity; and evaluation of the suitability of various organisms as assay organisms for selenium and its compounds.

Biological and chemical assay for hormones in living tissues and feed products continues in an effort to further clarify mode of action and effects of specific estrogens. Animals suffering from ingestion of toxic feeds are studied. New chemical and biological methods for testing and evaluating

non-nutritive additives in foods and feeds are being developed. For example, studies are being undertaken to isolate the thyroid stimulating factor(s) in food and feeds and to determine its chemical nature, its distribution and its metabolism.

The total State scientific effort reported as being devoted to pharmacology research is approximately 4.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Pharmacology Investigations

1. Citrus Products. The dihydrochalcones of bitter neohesperidin and naringin from citrus are intensely sweet and are potentially useful as low calorie sweeteners of food products and for use in sugar-free diets. Ninety-day feeding tests of rats were concluded, indicating no obvious toxicity at levels of ingestion up to 200 times the level calculated to be equivalent to the sweetening provided by the average daily sucrose consumption in man in the United States. The rats used in the 90-day feeding tests were also used for a reproduction assay. The safety of these compounds for use by man will depend upon the absence of unfavorable histopathological findings.

2. Deciduous Fruit. The enzyme O-methyl transferase has been found effective in preventing enzymic discoloration of cut and peeled fruit. Enzyme-substrate studies are under way in biological systems. A variety of ortho-diphenolic compounds, including phenolic acids, phenols, coumarin, and flavonoids were found to be suitable substrates for this enzyme.

3. Fermentation Product from Starch. Polysaccharide B-1459 is a fermentation product that offers considerable promise as a thickener. In low concentration it imparts high viscosity to water and aqueous solutions. It has the additional desirable property that it does not thin out when the solution is heated. It has potential application as a thickening agent for foods, pharmaceuticals and cosmetics, for fire fighting foams and for oil well drilling muds. As a potential food additive its toxicological safety is being evaluated. Ninety-day tests with male and female beagle dogs at levels of one to two grams per kilogram of body weight per day showed no untoward histopathological findings. Liver and kidney function tests were negative. However, the high level feeding resulted in a purgative action with some lowering of body weight, serum cholesterol, and hemoglobin and erythrocyte count.

Preliminary tests suggested the ability of this polysaccharide to lower serum cholesterol. Weanling rats were fed high cholesterol diets to which was added either polysaccharide B-1459 or a gum, pectin. Serum cholesterol was lowered in both instances but the polysaccharide had the greatest effect. Liver and fecal cholesterol determinations from these feeding tests are in progress.

In feeding tests with both rats and dogs, polysaccharide B-1459 was found to be an insignificant caloric source. In the case of rats, polysaccharide was

quantitatively excreted in the feces within the limits of experimental error. Carbon 14-labeled polysaccharide was fed to individual rats. Labeled-CO_2 appeared in expired air in less than two hours after presenting the diet and account for 2% of the total polysaccharide ingested during 24 hours. On autopsy, liver, kidney, and muscle were found to be labeled. However, in comparison with various plant gums, polysaccharide B-1459 is very resistant to degradation in the digestive tract.

4. Toxic Fescue. Further attempts to produce in experimental laboratory animals, symptoms that would correspond to the toxic responses in cattle ingesting toxic fescue have failed. Samples of toxic fescue extract and orchard grass extract were dissolved in sterile saline solution filtered and injected beneath the skin in Swiss mice at a level of 1 cc. per mouse per day for a period of eight days. No toxic results were observed in two weeks. Intravenous injection of 1/2 cc. of saline solution of the extracts administered on alternate days for two periods also failed to produce toxic symptoms after two weeks' observation. The extracts were found active in producing contraction of guinea pig ileum and uterus preparations. However, histamine releasing activity of the extracts was suspected and, when histamine release was blocked, the extracts had no effect on the uterus or ileum preparations.

Related studies, which had been conducted at Northwestern Medical School by Dr. A. C. Keyl before he joined the staff of the Western Regional Research Laboratory, indicated a light-sensitization factor in fescue toxicity. In those experiments mice with toxic fescue extract injected beneath the skin died in about a day if exposed to sunlight. Treated mice kept in the dark mostly survived but the survivors developed typical gangrenous tails seen in rats that receive ergot alkaloids. Samples of the toxic fescue and orchard grass extracts are now being tested on mice to see if the Northwestern Medical School results can be reproduced.

5. Cottonseed. Microbial and toxicity investigations have been conducted on cottonseed hulls, cottonseeds, and meal from moldy seeds. Aspergillus flavus was isolated from the moldy meal. Chromatographic evidence was obtained for the presence of aflatoxin from this meal, but spectroscopic identification was not successful due to difficulty of purification and losses encountered in chemical manipulations. However, the isolated Aspergillus flavus, when grown on shredded wheat biscuits, produced aflatoxin B as identified by the compound's behavior under ultraviolet light, chromatography and the ultraviolet spectrum of the purified material.

Rat feeding toxicity studies were conducted at dietary levels of 15% and 30% using a commercial cottonseed meal that had produced an 85% mortality in swine. After a 90-day feeding test, a reproduction assay was run. No adverse effects on food intake, growth, or reproduction were detected with rats fed this cottonseed meal at a 30% level. Histopathological studies are now being conducted on the tissues of these animals. It is doubtful that the toxic effects observed with the swine can be attributed to gossypol since

rats are quite sensitive to gossypol. The possibility of toxicity due to mold metabolites must be considered.

6. Cottonseed Oil. Halphen positive (cyclopropene-containing) cottonseed oil is known to exert an adverse effect on chickens and laying hens. However, there was no difference on growth and reproduction of rats fed Halphen positive and Halphen negative cottonseed oils at a dietary level of 15%. It could be tentatively concluded that a species difference between rats and chicks was involved. However, other factors may also be involved such as the validity of the chemical assay for the cyclopropene content of oil. Fatty livers were observed for both the Halphen positive cottonseed oil and the corn oil which was included in the diets of some animals for control purposes. Hence subsequent studies with an increased dietary level of choline were conducted, but the results were unchanged. Since other workers have reported toxic effects in rats fed cyclopropene containing oil from Sterculia foetida, it is tentatively concluded that the cyclopropene in the cottonseed oil we investigated was below the toxic level.

7. Dry Beans. Components of dried beans are under pharmacological investigation to seek information relating to intestinal distress caused by ingestion of cooked dry beans. One procedure with laboratory animals has been to inject suspect compounds or extracts into contiguous ligated intestinal segments, each having the same arterial count. Two substances included in a 60% ethanol extract of cooked beans were shown by direct observation to have a significant effect on the rat's small intestine. A buffered solution of S-methyl-L-cysteine was injected into one segment and an equal volume of buffer into the other. An hour later the ligated segments were excised and weighed. The experiment was repeated enough times to provide statistical significant data, indicating that S-methyl-L-cysteine caused an increase in intestinal segment weight due to mucous production. Authentic S-methyl cysteine and a dialysate from extracted cooked beans which was indicated by chromatographic procedures to contain S-methyl-L-cysteine, both caused contraction of a smooth muscle preparation. Histamine also caused such a contraction. An antihistaminic drug (Banadryl) inhibited the contraction of the smooth muscle preparation, leading to the conclusion that a histamine-releasing mechanism of action could be ascribed to S-methyl-cysteine.

8. Dried Fruit. Treatment of high-moisture dried fruits with ethylene oxide has been reported to result in residues of ethylene and diethylene glycol. Approval by Food and Drug Administration for use of ethylene oxide requires the development of an analytical method for the glycol residues, so they may be measured and controlled at nontoxic levels. It was demonstrated that when known amounts of ethylene glycol, the principal breakdown product of ethylene oxide and water, were added to high-moisture dried fruit that had not been treated with ethylene oxide, only 75-85% of the glycol could be recovered. A paper-chromatographic isolation technique has now been developed which makes it possible to quantitate the ethylene-oxide residues in high moisture dried dates.

9. Feed Values of Potential Replacement Crops.

Crambe abyssinica. Rat feeding tests with freshly ground dehulled Crambe seed resulted in toxicity when fed at levels of 5, 15, and 25% of the whole diet. All rats fed at the 25% level were dead in 42 days. Weight gains at the 5% dietary level were slightly below that of the control. Weight gain was reduced by a half for rats fed at the level of 15% of the diet. Ninety-day feeding tests were made with six other Crambe meal samples at a dietary level of 30%. One of these samples had been defatted and apparently retained all toxic constituents. Other samples were prepared by various processes in which thioglucosides were removed or modified by enzymatic conversion or enzyme inactivation. Livers from rats fed this material were abnormal in gross appearance and histological characterization.

Cassia species. Five species of Cassia were fed to rats at dietary levels of 5% for a period of 56 days. Two species compared favorably with the growth rate on a 5% soybean control diet.

Vernonia anthelmintica. Four samples of Vernonia marc were used for feeding tests. They included unautoclaved pericarp and kernel and autoclaved pericarp and kernel samples. The materials were fed at the 20% dietary level for 98 days. Good growth rate compared with control rats was observed for the autoclaved samples. Since unheated samples of the kernel fraction inhibited growth, a heat labile growth inhibitor must be involved. An obvious suspect, trypsin-inhibitor, could not be implicated since there was no pancreatic hypertrophy.

10. Estrogens from Forage Crops. Coumestrol is a compound in alfalfa and other forage crops that has estrogenic activity. The activity is measured biologically by an increase in the weight of immature mouse uterus from animals that have been fed the compound or feed containing the compound. Coumestrol has also been produced synthetically from flavylium salts, in connection with basic research on fruit anthocyanin pigments. The synthetic coumestrol and coumestrol extracted from alfalfa were shown to have identical biological activity. The availability of synthetic coumestrol made possible more extensive animal assay than was possible using the extracted material. Coumestrol in the diet produced temporary sterility in male mice with one gram of coumestrol per kilo of diet. In subsequent experiments, however, the level of coumestrol required to inhibit spermatogenesis in the male mouse was well above the level required to inhibit reproduction in the female.

11. Biodegradable Detergents. Acute oral toxicity of four alpha-phosphono fatty acids was determined in mice. Samples were relatively non-toxic, with dosages ranging from 2.5 to 3 grams per kilo body weight. Furthermore, all compounds tested proved to be non-irritating when applied to the shaved backs of rats. Skin irritant effects of 22 alpha sulfoesters of fatty acids were tested by topical application to rats of 2% solutions of each compound daily for 15 days. No evidence of skin irritation was observed for any of

the compounds during the 15 days of application and for one week after the applications were discontinued.

12. Caloric Availability Studies. Caloric availability assays were run on white wheat, bulgur, and puffed bulgur, using rats as test animals. Using invert sugar as the caloric standard, the three test materials were evaluated at 5.7, 5.1, and 4.6 calories per gram respectively. Digestibility of the three samples ranged from 89 to 91%.

13. Skin Irritation of Treated Cotton Cloth. Small squares of untreated and treated cloth, moistened with distilled water, were placed on the shaved skin of rabbits immobilized in stocks over night. After contact with the skin for 36 hours no evidence of skin irritation was produced by cloth treated with dimethylol methyl carbamate, dimethylol ethyl carbamate, and tris (N-methylol-2-carbamoylethyl) amine. Chronic feeding experiments are now in progress with these compounds, which are being fed to rats in order to evaluate their safety under these conditions.

14. Content and Availability of Fluorine in Chicken Bones. The poultry industry produces approximately 1 billion pounds of chicken backs and necks annually. Because of low market value of this material, it has been proposed that homogenized chicken backs and necks be incorporated in gravy, soup mixes, and new poultry products. Feeding tests with weanling rats were conducted to determine the availability of fluorine from ground bone meal prepared from dried chicken bones. Chicken bone meal was added to provide a range of dietary levels. For comparative purposes, identical levels of fluorine were provided in control weanling rat diets using sodium fluoride rather than the ground chicken bones. At the end of 190 days the results as measured by bleaching of rat incisor teeth were identical for the two sources of fluorine. These data support the contention that the fluoride in bone meal is as readily available as fluoride from sodium fluoride salt in the diet. The use of chicken back and neck bones for human food products must be evaluated relative to the total fluorine ingestion that is potential for any area or set of circumstances.

15. Castor Bean Allergens. Deallergenation of castor bean meal to a level where it is non-hazardous to castor allergic individuals can improve the prospects for development of castor as an important commercial agricultural crop. A successful deallergenation procedure based on steam cooking of castor bean pomace with dilute ammonium hydroxide was developed. The human allergic serum transfer test in non-human primates was used to demonstrate that allergy to castor beans can be cross-reacted to castor flour pollen and compounds from closely related plants such as the bull nettle and other spurges. However, allergic cross-reactions with compounds rich in chlorogenic acid, which has been suggested elsewhere, were not found following exhaustive laboratory test procedures. Marmosets were found to be useful laboratory animals for study of allergy by the allergic serum transfer test. Because these animals are less expensive and more easily kept in laboratories than macaques (a breed of Asian monkeys previously used), this finding should substantially advance research and clinical allergy studies following the developments reported here.

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Pharmacology Investigations

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1/ Research supported by P.L. 480 funds.

AREA NO. 14. REPLACEMENT CROPS-- UTILIZATION POTENTIAL

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available that would have new end-use patterns. For example, it would be advantageous to develop a new oilseed crop yielding fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species, followed by additional research to explore uses and demonstrate industrial potential, and by additional agronomic research to establish proper cultural practices and select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of pressure for greater national self-sufficiency, the nation will benefit from availability of practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potential. Work of the Department has already revealed several promising sources of new potentially valuable oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on by-products from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAM

Basic and applied research is being conducted on hydroxy-unsaturated acid-containing oilseeds, in the Western Utilization Research and Development Division's headquarters laboratory at Albany, California; and by contract at Fargo, North Dakota. The basic, compositional studies emphasize the development of special analytical techniques for application to new oils containing hydroxy-unsaturated fatty acids. In the applied area, research is conducted to develop and evaluate industrial products from the hydroxy-unsaturated oils.

The Federal program of research in this area totals 4.2 professional man-years, including contract research at a rate equivalent to approximately 0.2 professional man-years per year. Of this total, 2.6 are assigned to chemical composition and physical properties; and 1.6 to industrial utilization.

PROGRAM OF STATE EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, drugs, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention.

Basic aspects of this program involve study of the biochemical and physiological bases for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms.

Horticultural specialty crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 9.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Dimorphotheca and Lesquerella Seed Oils. Oils of Dimorphotheca sinuata and wild members of the mustard family, the Lesquerellas, are being isolated, purified, and analyzed. Analysis by far ultraviolet provides a useful tool for identification of unsaturated fatty acids from these and other oils. Constitutional information and preparative and purification methods were advanced as preliminary steps to finding profitable uses for Lesquerella and Dimorphotheca oils. Methyl esters of separated fatty acids from these oils were synthesized to provide starting materials for new derivatives which will be screened for industrial utilization.

B. Industrial Utilization

1. Industrial Products from Hydroxy-Unsaturated Oils. Large lots of L. densipila, L. gordonii, and L. fendleri oils were processed, converted by

base-catalyzed methanolysis, and purified by fractional distillation at reduced pressure. Methyl lesquerolate was pyrolyzed to methyl 12-tridecenoate which was converted in high yield to 13-amino tridecanoic acid. This amino acid is the building block for nylon-13. Improved techniques were developed for alkaline cleavage of the conjugated hydroxy systems in fatty acids from these seed oils. The hydroxy acids obtained should yield interesting linear polymers. In contract research at North Dakota State University good clear varnishes were developed from isocyanate-modified Dimorphotheca oils. Properties of films made from dehydrated castor and Lesquerella oils were evaluated. Films produced from dehydrated Lesquerella oil and castor oil showed some after-tack, and the Lesquerella oil films were somewhat softer than those of castor oil. Modification in the treatment of these oils and their derivatives is continuing and the relationship of cis-trans isomer content to after-tack is being evaluated. Combinations of hydroxy unsaturated oils under investigation with other diisocyanates will be evaluated for use in surface coatings.

Vinyl esters of 9-chloro and 9-hydroxystearic acid and 14-chloro and 14-hydroxyeicosanoic acid were prepared, purified, and characterized here and their co- and homo-polymerization studied by the contractor. The polymers will be evaluated for industrial value.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

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Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress (Yes-No)	Incl. in Area and Subheading
W1 2-24 (Rev.)	Air classified flours	Albany, Calif.	Yes	1-B-2 1-B-4
W1 2-27 (Rev.)	Wheat feed products	Albany, Calif.	Yes	1-B-5 1-C-1
W1 2-29 (Rev.)	Bread flavors	Albany, Calif.	Yes	1-A-8
W1 2-31[1]	Effects of processing on properties and new product development	Albany, Calif.	Yes	2-A-1 2-B-1 2-B-2
W1 2-41	Wheat endosperm constituents	Albany, Calif.	Yes	1-A-3 1-A-5
W1 2-42 (C)[1]	Methods for determining wheat proteins	Pullman, Wash.	Yes	1-A-3
W1 2-43 (Rev.)	Gluten foods	Albany, Calif.	Yes	1-B-2
W1 2-44	Protein interactions	Albany, Calif.	Yes	1-A-1 1-B-4
W1 2-45 (C)	Water-dispersible protein preparations	Lafayette, Indiana	Yes	1-B-2
W1 2-46 (C)	Chemical basis for cohesiveness in gluten	Kansas City, Missouri	Yes	1-A-4
W1 2-47 (C)	Elimination of microbial contaminants of wheat flour	Chicago, Ill.	Yes	1-B-6
W1 2-48 (C)	Identification of wheat proteins by radiotracer techniques	Pullman, Wash.	Yes	1-A-3
W1 2-49 (C)	Protein and lipid composition of spring and winter wheat	Manhattan, Kansas	Yes	1-A-6
W1 2-50	Mechanism of flour maturation	Albany, Calif.	Yes	1-A-6
W1 2-51	Compositional factors of wheat relative to continuous-mix processes	Albany, Calif.	Yes	1-A-1
W1 2-52 (C)	Wheat bran and aleurone pigments	Corvallis, Oregon	No[2]	
W1 2-53	Protein-rich fractions from mill run	Albany, Calif.	Yes	1-B-2
W1 2-54	Light-colored bulgur for specific markets	Albany, Calif.	Yes	1-B-1 1-B-5
W1 2-55 (Gr)	Oxidation-reduction enzymes	Madison, Wisc.	No[2]	
W1 2-56 (C)	Rheological study of doughs	Palo Alto, Calif.	No[2]	
W1 2-57 (C)	Carrying capacity of HRW wheats	Manhattan, Kansas	No[2]	
W1 2-58 (C)	Protein changes during malting	Minneapolis, Minnesota	No[2]	
W1 3-16 (Rev.2)	Improved forage feed products	Albany, Calif.	Yes	3-B-2
W1 3-18	Phenolic components of forages	Albany, Calif.	Yes	3-A-1 3-A-2 3-B-1 3-B-2
W1 3-19 (C)	Autoxidation of alfalfa lipids	Berkeley, Calif.	Yes	3-A-3
W1 3-20 (C)	Products from southeastern grasses	Athens, Georgia	No[2]	
W2 2-7 (Rev.2)	Molecular properties of wool and mohair proteins	Albany, Calif.	Yes	4-A-1
W2 2-11 (Rev.)[1]	Minimizing degradation of wool by acids and alkali	Albany, Calif.	Yes	4-A-1 4-B-5

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

Line Project Check List -- Reporting Year July 1, 1963 to June 30, 1964

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W2 2-22 (Rev.)	Chemical treatment of wool for shrink resistance and other "easy-care" properties	Albany, Calif.	Yes	4-B-1 4-B-2 4-B-4
W2 2-24	Effect of fabric construction and functional properties	Albany, Calif.	Yes	4-B-3
W2 2-28 (Rev.)	Mechanical behavior of wool fibers and fibrous assemblages	Albany, Calif.	Yes	4-A-2 4-A-3
W2 2-29 (Rev.)	Effects of radiation on natural and modified wools	Albany, Calif.	Yes	4-A-3
W2 2-30[1]	Nuclear magnetic resonance absorption of natural and modified wool and mohair	Albany, Calif.	Yes	4-A-1
W2 2-32	New types of yarns and fabrics from coarse wools	Albany, Calif.	Yes	4-B-1
W2 2-33 (C)[1]	Improved bleaching of wool	Lowell, Mass.	Yes	4-B-5
W2 2-34 (C)	Chemical modification of wool to increase drying rate	Durham, North Carolina	Yes	4-A-1
W2 2-35 (C)	Wear-wrinkling performance of light weight wool fabrics	Washington, D. C.	Yes	4-B-3
W2 2-36	WURLAN treatment of wool top	Albany, Calif.	Yes	4-B-1
W2 2-37 (C)	High luster wool fabrics	Washington, D. C.	No[2]	
W3 1-67 (Rev.2)[1]	Processing quality of Northwest soft fruit and berry varieties	Prosser and Puyallup, Wash.	Yes	6-B-5
W3 1-83 (Rev.)	Flavonoids in citrus	Pasadena, Calif.	Yes	5-A-2 5-B-1
W3 1-88 (Rev.)	Citrus essential oils	Pasadena, Calif.	Yes	5-A-1 5-B-1
W3 1-95 (Rev.)[1]	Composition of dates and date products	Pasadena, Calif.	Yes	5-A-5 5-B-2
W3 1-101 (Rev.)	Fruit juice products and processes	Albany, Calif.	Yes	6-B-6
W3 1-109 (Rev.)[1]	Processing quality of Northwest grapes for juice	Prosser, Wash.	Yes	6-B-4
W3 1-112 (Rev.)	Dried fruit products and processes	Albany, Calif.	Yes	6-A-2 6-A-4 6-B-1
W3 1-117 (Rev.)	Fruit pigments	Albany, Calif.	Yes	6-A-1
W3 1-119	Fruit flavor components	Albany, Calif.	Yes	5-A-4 5-B-4 6-A-5
W3 1-120 (C)	Macadamia nuts products and processes	Honolulu, Hawaii	Yes	6-B-7
W3 1-121	Heat transfer surface fouling	Albany, Calif.	Yes	6-B-6 8-B-1
W3 1-122	Texture of fruits and fruit products	Albany, Calif.	Yes	6-A-3 6-B-3
W3 1-123 (C)	Fruit leucoanthocyanins	Los Angeles, Calif.	Yes	6-A-1
W3 1-124 (Gr)	Cell wall organization of fruits	Cambridge, Mass.	Yes	6-A-3
W3 1-125	Composition of desert grapefruit	Pasadena, Calif.	Yes	5-A-1
W3 1-126	Viniferous grape products	Albany, Calif.	Yes	6-A-5 6-B-4
W3 1-127	New fruit dehydration methods	Albany, Calif.	Yes	6-B-2
W3 1-128	Rancidity control in walnuts	Pasadena, Calif.	Yes	6-B-7

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W3 1-129 (C)	Grape juice extraction	Davis, Calif.	No[2]	
W3 1-130	Date composition and products	Pasadena, Calif.	Yes	5-A-5 5-B-2
W3 1-131	Tropical fruit products	Honolulu, Hawaii	Yes	5-B-3
W3 1-132	Processing quality--Northwest fruits	Prosser and Puyallup, Wash.	Yes	6-B-5 6-B-6
W3 1-133 (C)	Products from desert grapefruit	Tucson, Arizona	No[2]	
W3 1-134 (C)	Phenolics in canned apple juice	Fort Collins, Colorado	No[2]	
W3 4-47 (Rev.)[1]	Vegetable varieties	Prosser and Puyallup, Wash.	Yes	8-B-4
W3 4-74 (Rev.)	Composition of dry beans re processing factors and product quality	Albany and Pasadena, Calif.	Yes	8-A-3 8-A-4 8-A-5 8-B-2
W3 4-75[1]	Measurement and preservation of chlorophyll in vegetables	Albany, Calif.	Yes	8-A-6 8-B-3
W3 4-77 (Rev.)	Tomato concentrate and powder	Albany, Calif.	Yes	8-B-1 8-B-4
W3 4-79 (Rev.)	Effects of processing on potato product flavor	Albany, Calif.	Yes	7-A-1 7-B-1
W3 4-80	Effects of processing operations upon texture of frozen vegetables	Albany, Calif.	Yes	8-A-7 8-B-3
W3 4-81 (C)	Dry bean characteristics	Urbana, Ill.	Yes	8-A-3
W3 4-82	Microbiology of frozen vegetables	Puyallup, Wash.	Yes	8-B-5
W3 4-83	Mechanism of anhydration in bacterial spores	Albany, Calif.	Yes	8-A-2
W3 4-84 (C)	Histological studies of vegetables for dehydration	Davis, Calif.	Yes	8-A-7
W3 4-85	Flavor of tomato products	Albany, Calif.	Yes	8-A-1
W3 4-86	Chemistry and enzymology of vegetable flavors	Albany, Calif.	Yes	8-A-1
W3 4-87 (C)	Removal of radioactive fallout	Berkeley, Calif.	No[2]	
W3 4-88	Processing quality--Northwest vegetables	Prosser and Puyallup, Wash.	Yes	8-B-4
W3 4-89 (C)	Dry bean and pea powder	East Lansing, Michigan	No[2]	
W3 4-90 (Gr)	Sporulation of food spoilage bacteria	Urbana, Ill.	No[2]	
W4 3-1	Chemical derivatives of ricinoleic acid	Albany, Calif.	Yes	9-A-2
W4 3-2 (Rev.)	Foamed polyurethanes from castor oil	Albany, Calif.	Yes	9-B-1
W4 3-3 (Rev.)	Pharmacology of castor bean allergens	Albany, Calif.	Yes	9-A-1 9-B-2
W4 3-5 (C)	Polymerization of castor oil-derived monomers	Tucson, Ariz.	Yes	9-B-1
W4 3-6	Role of blossoms and pollen in castor allergy	Albany, Calif.	Yes	9-A-1
W4 3-7 (C)	Characterization of antigenic proteins of castor seed	Menlo Park, Calif.	No[3]	
W4 3-8	Castor pomace deallergenation	Albany, Calif.	Yes	9-B-2
W4 3-9	Safflower oil and meal	Albany, Calif.	Yes	9-A-2

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

[3] Investigation has been terminated without completion of the contract. Final negotiations are pending.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
W5 1-72 (C)[1]	Relation of beet composition to processing characteristics	Fort Collins, Colorado	No[4]	
W5 1-73	Biochemical studies of non-sucrose carbohydrates in sugar beets	Albany, Calif.	Yes	10-A-1
W5 1-75	Effects of non-sugar chemicals on processing	Albany, Calif.	Yes	10-A-1 10-B-1 10-B-2 10-B-3
W5 5-37	Evaluation of hydroxy-conjugated dienoic acid oils	Albany, Calif.	Yes	14-A-1
W5 5-46 (C)	Preparation and evaluation of surface coatings	Fargo, North Dakota	Yes	14-B-1
W6 1-41 (Rev.)	Improvement of egg white products	Albany, Calif.	Yes	12-B-1
W6 1-48 (Rev.)	Chemistry of poultry flavor	Albany, Calif.	Yes	11-A-2
W6 1-49 (Rev.)	Microbiology of cold-tolerant organisms	Albany, Calif.	Yes	11-B-2
W6 1-53[1]	Processing characteristics of eggs	Albany, Calif.	Yes	12-B-1
W6 1-54	Precooked frozen foods	Albany, Calif.	Yes	12-B-3
W6 1-55	Improvement of yolk-containing egg solids	Albany, Calif.	Yes	12-B-2
W6 1-56	Tenderness and other textural qualities of poultry meat	Albany, Calif.	Yes	11-A-1
W6 1-57 (C)[1]	Oxidative changes in yolk lipids	Austin, Minn.	Yes	12-A-1
W6 1-58 (C)	Control of the neuromuscular retention and release of feathers	East Lansing, Michigan	Yes	11-A-4
W6 1-59 (C)	Reduction of Salmonella contamination in egg products	Ames, Iowa	Yes	12-B-1
W6 1-60 (C)	Histological study of frozen poultry	Madison, Wisc.	No[2]	
W6 1-61	Elimination of Salmonella in egg products	Albany, Calif.	Yes	12-B-1
W6 1-62	Freeze-dried poultry meat	Albany, Calif.	Yes	11-B-1
W6 1-63[1]	Content and dietary availability of fluorine in chicken bones	Albany, Calif.	Yes	11-A-3
W6 1-64 (Gr)	Salmonella metabolism	Ithaca, New York	No[2]	
W6 1-65 (C)	Freeze drying of poultry meat	Berkeley, Calif.	No[2]	
W6 1-66 (C)	Pasteurization of eggs	Davis, Calif.	No[2]	
WU-P-1	Plant enzymes	Albany, Calif.	Yes	6-A-6
S4 1-115 (C)	Ethylene copolymerization with unsaturated fatty acids	Tuscola, Ill.	No[2]	
WU-0-0-1(BF)	Hop oil flavor components	Albany, Calif.	Yes	8-A-1
WU-0-0-2(OCD)	Fallout shelter foods	Albany, Calif.	Yes	1-A-8 1-B-3
UR-A10-(10)-22	Rheology of wheat flour doughs	Haifa, Israel	Yes	1-A-2
UR-E9-(10)-2	Composition of whole wheat lipids	Paris, France	Yes	1-A-7
UR-E9-(10)-7	Immunochemical analysis of wheat and barley proteins	Paris, France	Yes	1-A-3 1-A-5

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

[4] Final progress reported last year.

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UR-E9-(10)-8	Solubility of wheat gluten proteins	Montpellier, France	No[5]	
UR-E9-(10)-43	Phosphorus in wheat flour	Paris, France	Yes	1-A-7
UR-E9-(10)-44	Ultrasonic study of wheat gluten	Paris, France	Yes	1-A-3
UR-E9-(10)-45	Enzyme action in low-moisture grain	Paris, France	Yes	1-A-5
UR-E15(10)-31	Wheat germ proteins	Bologna, Italy	Yes	1-A-3
UR-E21-(10)-1	Sulfhydryl groups in wheat	Poznan, Poland	Yes	1-A-5
UR-E21-(10)-18	Coenzyme role of riboflavin of wheat endosperm	Poznan, Poland	Yes	1-A-5
UR-E29-(10)-14	Wheat flour lipids	Chorleywood, England	Yes	1-B-4
UR-E29-(10)-38	Separation of the total protein of wheat flour	St. Albans, England	Yes	1-A-3
UR-E29-(10)-47	Biological value of processed wheat	Cambridge, England	Yes	1-B-5
UR-01-(10)-1	Dough rheology	North Ryde, Australia	Yes	1-A-2
UR-E8-(10)-15	Enzymes in forages	Helsinki, Finland	No[2]	
UR-E15-(10)-17	Natural antioxidants in alfalfa	Milano, Italy	No[2]	
UR-E29-(10)-52	Structure of alfalfa polysaccharides	Edinburgh, Scotland	Yes	3-A-4
UR-A7-(10)-15	Molecular processes in wool	Allahabad, India	No[2]	
UR-E8-(20)-10	Finishing treatments for improved qualities in wool fabrics	Helsinki, Finland	Yes	4-B-6
UR-E9-(20)-1	Sequence of amino acids in wool proteins as related to quality differences	Lille, France	Yes	4-A-1
UR-E26-(10)-7	Sulfur in wool keratins	Stockholm, Sweden	No[2]	
UR-E29-(20)-11	Penetration of charged molecules into keratins	Leeds, England	Yes	4-A-1
UR-E29-(20)-22	Lubrication of wool knitting yarns	Nottingham, England	Yes	4-B-3
UR-A7-(10)-60	Fruit leucoanthocyanins	Delhi, India	Yes	6-A-1
UR-A10-(30)-32	Enzymatic browning in deciduous fruits	Jerusalem, Israel	Yes	6-A-2
UR-S5-(10)-2	Tropical fruit flavors	Bogota, Columbia	No[2]	
UR-A7-(10)-39	Bean proteins	Allahabad, India	No[2]	
UR-E9-(10)-54	Spore enzymes	Paris, France	No[2]	
UR-E26(30)-5	Autoxidation of fats in dehydrated vegetables	Gothenburg, Sweden	Yes	7-A-1
UR-E29-(30)-16	Enzymatic browning of potato	Cambridge, England	Yes	7-A-2
UR-E29-(30)-17	Sulfur dioxide in dehydrated vegetables	London, England	Yes	7-A-3

[2] Recently initiated project.

[5] Delays in obtaining equipment and personnel changes prevented advance of this project during the reporting period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress (Yes-No)	Area and Subheading
UR-E29-(30)-20[1]	Carotenoid components of vegetables	Cambridge, England	Yes	8-A-6
UR-E29-(30)-27	Relationship of composition to cooking quality of dry peas	Chipping-Campden, England	Yes	8-A-4
UR-A7-(10)-21	Hydroxylated derivatives of vegetable oils	Hyderabad, India	No[2]	
UR-A7-(50)-31	Reaction of sucrose with sulfonyl chloride and other chemicals	Calcutta, India	Yes	10-B-3
UR-A10-(50)-25	Enzymatic sucrose degradation in sugar beet tissues	Jerusalem, Israel	No[6]	
UR-E29-(50)-33[1]	Fatty acid esters of sucrose	London, England	Yes	10-B-3
UR-A7-(60)-27	Physico-chemical properties of hen egg yolk proteins caused by freezing	Bangalore, India	Yes	12-A-2
UR-E9-(60)-76	Chemistry of egg lysozyme	Paris, France	Yes	12-A-2
UR-01-(10)-4	Ovalbumin in eggs	Ryde, New South Wales, Australia	Yes	12-A-2

[1] Project discontinued during the reporting period.

[2] Recently initiated project.

[6] No progress reported in 1964.